THE INFLUENCE OF SIMAZINE ON METABOLISM OF PROTEIN AND RIBONUCLEIC ACID IN OAT PLANTS (*Avena sativa* L.)

By

RAGHVENDRA PAL SINGH

UNIVERSITY OF FLORIDA

December, 1964

ACKNOWLEDGMENTS

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Weeds are among the most serious obstacles in
the production of crops. The science of weed control
has undergone more advancement during the last two decades
than in the past centuries of recorded history. Old
methods of weed control are being replaced with chemical
techniques that provide far superior control at reduced
cost.

According to the British Weed Control Council (16),
71 herbicides were named in 1960. Yet, in actual practice,
some three quarters of all chemical weed control is now
achieved with just three chemicals, their homologues or
analogues. One of these chemicals is simazine (2-chloro-4,
6-bis(ethylamino)-$\underline{s}$-triazine), an effective, selective,
pre-emergence herbicide for weed control in corn.

The knowledge of the influence of herbicides on
physiological processes and the biochemical constituents
of plants is useful in the understanding of the mode of
action of herbicides and developing efficient weed control
practices. In the past years, it has been established
that simazine inhibits photosynthesis by influencing the
Hill reaction (34, 96) and CO_2 fixation (8, 130).

1

As yet, no attempt has been made to understand the influence of simazine on protein and ribonucleic acid (RNA) metabolism of plants. Protein and nucleic acid metabolism are essential processes which greatly influence growth and development of plants.

This investigation was designed, therefore, to explore the influence of simazine on protein and RNA metabolism of oat plants. Secondary objectives included the determination of the influence of simazine on plant growth and the content of chlorophyll and free amino acids. Radioisotope C^{14}-leucine and P^{32} were used in this investigation to gain a better understanding of the influence of simazine on the synthesis of protein and RNA.

REVIEW OF LITERATURE

Simazine and atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) have proven to be outstanding chemicals for weed control in corn (19, 47, 83). Simazine was synthesized by Gysin and Knüsli in the laboratory of J. R. Geigy, Basle, Switzerland and was released for experimental purposes in 1956 (93). Simazine is a selective pre-emergence herbicide effective in the control of broad leaf weeds and annual grasses (100). The most common weeds controlled by this herbicide, are carpet weed, wild carrot, wild onion, lambsquarter, narrow-leaved plantain, purslane, rag weed, foxtailgrass, barnyardgrass, quackgrass and some other annual grasses (18, 47).

Greenhouse and field studies indicated that tall fescue and alfalfa forage crops are the most tolerant to atrazine, timothy is the most susceptible and red clover, Korean lespedeza, ladino clover, sweetclover, orchardgrass and bromegrass are intermediate in their response to atrazine (36).

Sugarcane, brambles, grapes, asparagus, strawberries, pineapple, citrus, milo, spinach and walnut were found to be tolerant to simazine (93). Cotton response is intermediate and cucumber, oats, barley and wheat are susceptible to simazine (19).

3

Uptake and Translocation

Many of the less water-soluble herbicides of the
s-triazine group are effective only when absorbed by the
roots. Simazine apparently penetrates the cuticle readily
but subsequently moves only in an acropetal direction by
diffusion along cell walls and does not enter readily in
the living symplast (25). Other s-triazines follow the
same pattern of distribution when applied to the leaves (25).

These compounds are readily absorbed by roots
from the culture medium (28, 93). Biswas (14) found that
atrazine moves both acropetally and basipetally, whereas
simazine moves only in acropetal direction. Simazine is
translocated in the acropetal direction in association
with the transpiration stream (28, 38, 39, 50, 95). The
foliar absorption and translocation of simazine in the
symplasm of corn, soybean, cucumber and cotton were found
to be negligible (28, 38, 39).

A significant difference in the rate of atrazine-C^{14}
absorption by the upper and lower surfaces of peanut leaves
was demonstrated by Biswas (14).

Simazine or C^{14}-labeled products, according to
Davis et al. (28), are translocated from roots to leaves in
cucumber in less than 0.5 hour. The radioactivity was first
confined to the vein but after eight hours, the herbicide
accumulated in the leaf margins and interveinal areas.
Simazine was distributed throughout oat seedlings within
three hours (95) and within 6-12 hours in seedlings of
several evergreen and deciduous tree species (50).

Light, temperature, relative humidity and the
concentration of herbicide influenced simazine absorption
(84, 95). Increased uptake and translocation of simazine-C^{14}
in corn under constant light had been reported by Plaisted
and Ryskiewich (84). Sheets (95) found more absorption
and translocation of simazine in oat seedlings at 37°C
and 33 per cent relative humidity than at 26°C and 66 per
cent relative humidity.

Various loci for accumulation of degraded products
of simazine-C^{14} in different plants have been reported by
Davis et al. (28). These products accumulated in the
edges of cucumber leaves, in the lysigenous glands of stem
and in the leaf edges of cotton and in the entire corn
plant. Foy (37) also reported its accumulation in the
lysigenous glands of cotton. He did not find an accumulation
of 2-hydroxy simazine in this gland.

Degradation of Herbicide

Corn is tolerant to s-triazine herbicides due
to detoxication of the herbicide. Studies with ring-
labeled simazine have demonstrated that both tolerant and
susceptible species degrade simazine, particularly in the
roots (21, 70, 96, 100). Simazine is dechlorinated to its
2-hydroxy analogue by a non-enzymatic detoxication
mechanism (22, 51). The degraded product, 2-hydroxysimazine
(2-hydroxy-4,6-bis(ethylamino)-s-triazine) has been isolated
from maize seedlings and excised roots (54).

Roth (90) found a factor in maize extracts which was not present in wheat extracts and was heat-labile in crude extracts, but heat-stable after purification. This factor was identical to a compound from maize identified as 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (53). The compound from maize and its glucoside converted simazine to hydroxysimazine in vitro (54).

In addition, other chemical and physiological systems may be involved in simazine detoxication.

1. Castelfranco and Brown (21) observed that hydroxy-lamine or pyrimidine converted simazine to its 2-hydroxy analogue in vitro.

2. Roth (91) observed that simazine is degraded by phenols and peroxidase. Resistant plants had high peroxidase activity and low catalase activity in comparison to susceptible species. A phenol-peroxidase system capable of breaking the heterocyclic nucleus of triazine has been obtained from maize. Both resistant and susceptible plant species cleaved the triazine ring of ring-labeled simazine (70, 85) and atrazine, and released $C^{14}O_2$. Initial release of $C^{14}O_2$ was higher from susceptible cucumber than from maize, but the chloroform-soluble fraction (probably unaltered simazine) was also higher in cucumber (85).

In general, the selective action of simazine seems to be associated with processes which prevent accumulation of the unaltered molecule at the site of action in the leaves (28, 96). However, Gysin and Knüsli (51) found accumulation of a considerable amount of unaltered simazine in a resistant plant.

Recently, Hamilton (52) found a single gene honozygous recessive mutant in an S_1 inbred line of corn which resulted in an 8-10 fold reduction in 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (simazine resistant factor) content. Tolerance studies on 3:1 and 1:1 segregating ears

indicated that the low segregates are somewhat less tolerant
to atrazine. Eastin et al. (33) found G.T.112 line of corn
to be susceptible to simazine and atrazine.

Injury Symptoms

The typical symptom of simazine toxicity is
chlorosis which starts at the leaf tip and progresses
along the margins to the base of the leaf. Necrosis of
the tissue occurs in the chlorotic area. Chlorosis spreads
rapidly to the entire leaf surface followed by death of
the leaf and eventually the entire plant. The death of the
entire plant results, particularly if enough moisture is
present to leach the chemical to root depth (93). Rodgers
and Wilcox (89) found that the phytotoxicity symptoms of
triazines in Lakeland fine sandy soil are evident 7 to 10
days after planting as leaf chlorosis in oats and leaf
chlorosis, stem binding and breaking in cucumber. These
plants died 14 days after planting.

Influence on Plant Anatomy

Ashton et al. (5, 6) studied the structural changes
in Phaseolus vulgaris induced by atrazine. These workers
observed the following histological changes:

1. Precocious development of vacuoles and a modified
ontogeny of chloroplast in the developing leaves.

2. Chloroplast destruction and reduced airspace system
in the matured primary leaves.

3. Cessation of cambial activity and decrease in the
thickness of the cell walls of sieve and tracheary elements
in the stem.

They studied these changes in simazine treated plants under light and dark conditions, and in the control plants placed in dark and light. They concluded that the accelerated vacuolation effect probably is due to the block in photosynthesis, but the chloroplast disintegration, reduced airspace system and cessation of cambial activity are not dependent exclusively on the photosynthetic block.

Ashton et al. (6) also studied the effect of atrazine on the fine structure of chloroplasts. The following results were observed:

1. Chloroplasts become spherical in form rather than discoid.

2. Then they become aggregated in the equatorial zone of the cell.

3. Eventually the individual chloroplasts disintegrate and the entire cell is filled with a substance which has a great affinity for a certain dye.

4. The ectoplast and tonoplast are modified.

In addition, Ashton et al. (6) have shown that chloroplasts from plants treated with atrazine and kept in the light undergo the following changes:

1. Starch disappears from the lamellar system.

2. The frets or part of the frets are destroyed, and further grana arrangement is disorganized.

3. Progressive swelling of the grana compartments.

4. In the late stages of the effect, the chloroplast envelope and compartment membranes are broken.

Wakonig and Arnason (111) have reported that 2,4,6-tris(ethylamino)-1,3,5-triazine caused chromosome breaks and chromatid interchange in the root tips of Vicia and Allium.

Physiological and Biochemical Effects

Interaction of light and triazines: Atrazine and
simazine are active only in the presence of light (2).
Ashton (3) found characteristics leaf injury symptoms of
atrazine to red kidney and oat plants only in the presence
of light. Less injury was observed at lower light
intensities. The action spectrum corresponded to the
absorption spectrum of chlorophyll. The nature of this
interaction of atrazine and light to produce phytotoxicity
is not known. However, Ashton et al. (6) postulated the
possibility of the formation of one or more secondary
toxic substances responsible for this effect. They also
suspected that one or more "free radicals" of this
substance may exist.

The results of the various investigations reveal
that photosynthesis is the process which is most sensitive
to phytotoxic effects of triazines. Several investigators
have demonstrated that simazine inhibits starch (45),
sucrose-C^{14} formation in leaves (8), oxygen release in
Elodea (91) and CO_2 utilization by illuminated bean leaves
(8).

Hill reaction: Simazine inhibits the photochemical
activity of isolated chloroplasts, according to many
investigators. All the active herbicides of the s-triazine
group gave 50 per cent inhibition of the Hill reaction at
concentrations between 10^{-4} and $10^{-9}\underline{M}$ with the 50 per cent
inhibition at a simazine concentration of $7 \times 10^{-9}\underline{M}$ (34, 35).

Sheets (96) reported that a $4.6 \times 10^{-6} \underline{M}$ simazine concentration caused the inhibition of the Hill reaction. Moreland (71) also found 50 per cent inhibition for isolated barley chloroplasts at $4.6 \times 10^{-6} \underline{M}$ simazine concentration. The simazine concentration required for 50 per cent inhibition of the Hill reaction had been established by Moreland and Hill (72).

According to Roth (91), triazines inhibit photosynthesis but increase oxygen consumption in respiration in green plants.

Good (48) and Bishop (13) concluded that inhibition results from interference with the mechanism of oxygen production. All phytotoxic triazines seem to inhibit the Hill reaction, but the degree of inhibition does not always parallel the herbicidal effectiveness (51). Hill reaction activity of chloroplasts from simazine-sensitive and simazine-tolerant species is inhibited equally (34, 71, 72) and the mechanism controlling selectivity consequently seems to be located outside the chloroplasts.

CO_2 fixation: Photosynthesis is inhibited in simazine-resistant species, but van Oorschot and Belksma (109) showed that CO_2 utilization by maize returns to normal within a few weeks after treatment.

Ashton et al. (8) reported the effects of different concentrations and exposure periods of simazine on CO_2 fixation by bean plants. After 40 hours of exposure

to 0.25 p.p.m. simazine concentration, the CO_2 fixation decreased to 30 per cent. The CO_2 fixation was nearly blocked when the tissue was treated with 1 p.p.m. simazine. In the exposure period study, CO_2 fixation was inhibited after an exposure period of three hours.

Zweig and Ashton (130) treated excised bean leaves with varying concentrations of atrazine for different time periods. The CO_2 fixation was normal at all exposure times when tissue was treated with 0.1 p.p.m. atrazine. At 1 p.p.m., CO_2 fixation was reduced to 10 per cent after 50 hours of exposure. A higher concentration of atrazine was more effective in the inhibition of CO_2 fixation. Results of these workers showed that atrazine could not completely block CO_2 fixation, even at relatively high concentrations and long exposure. In the above experiment, 1 per cent CO_2 fixation was found when the tissue was exposed 100 hours to 10 p.p.m.

According to Zweig and Ashton (130), atrazine treatment greatly modifies the course of organic compound synthesis in bean leaves. The results of their experiment demonstrated that sucrose synthesis had been completely inhibited by atrazine, but that the concentration of aspartic acid had increased and glycine disappeared. Citric acid, serine and asparagine also were affected. These results indicated that atrazine affects mostly the products of tricarboxylic acid cycle, which suggests that respiration and dark CO_2 fixation resembled the pattern of leaves

treated with 10 p.p.m. atrazine for 72 hours. Aspartic
acid was greatly increased and malic acid, serine,
glutamic acid and asparagine were present only in detec-
tible amounts, but sucrose-C^{14} was completely lacking.
They concluded that the disappearence of the products of
photosynthesis (sucrose, glyceric acid) and labeling of
aspartic acid and malic acid are due to the phosphoenol
carboxylase activity.

The $C^{14}O_2$ fixation in the atrazine treated
leaves was compared by Zweig and Ashton (130) with the
untreated leaves under dark treatment. They found that
the C-1 carboxyl label of aspartic acid represented 64
per cent of the total activity in the atrazine treated
leaves and 57 per cent in the dark treatment. On the
basis of these findings, these investigators concluded
that the same mode of $C^{14}O_2$ fixation is operative in
both leaves.

Carbohydrate: The control of quackgrass by atrazine
has been correlated by Schirman and Buchholtz (92) with
the depletion of carbohydrate in the rhizomes. Several
experiments have been carried out to illustrate the
effects of feeding of sugar and organic acids on the
toxic effects of simazine and atrazine. Glucose supplied
to barley seedlings was effective in keeping plants alive
in the presence of lethal concentration of simazine (71).

Ashton and Uribe (7) studied effect of feeding
sucrose-C^{14} to atrazine treated excised bean leaves on the

distribution of radioactivity in various labeled compounds.
In the presence of sucrose-C^{14} substrate and light, atrazine
treatment decreased serine, alanine and glyceric acid
biosynthesis and increased aspartic acid and glutamic
acid biosynthesis. Similar results were obtained in the
treatment where check plants were grown in the dark. The
only exception in these treatments was that the alanine
and glutamic acid were not affected in the dark. When
serine-C^{14} was used as substrate in the above condition,
atrazine treatment decreased malic acid and citric acid
biosynthesis and increased alanine, threonine, aspartic
acid and glutamic acid biosynthesis. Results under light
and dark conditions were similar.

        Allen and Palmer (2) reported that the toxic
effects of simazine can be overcome by feeding sucrose,
glucose, aspartate and maltose to plants. More soluble
carbohydrates were in the glucose and maltose fed shoots.
Plants fed with glutamine and asparagine were high in
total nitrogen. The nitrogen content of the sucrose and
maltose fed plants was increased, but nitrogen content
was not increased in the plants fed with glucose.

    Transpiration: A severe reduction in transpiration
rate is caused by the treatment of atrazine and other
inhibitors of photosynthesis. Smith and Buchholtz (101)
attributed this reduction to stomatal closure. The
stomates are closed due to the inhibition of photo-
synthesis. Wills et al. (121) also reported reduced

transpiration both in intact and excised shoots of corn,
cotton and soybean. They found high moisture content in
treated plants up to 24 hours after treatment, but this
moisture content later dropped down.

Respiration as well as the activity of catalase,
peroxidase, phenol oxidase, ascorbic acid oxidase and
glycolic acid oxidase had been decreased by atrazine,
according to Funderburk and Davis (43), in oats, soybean,
bean, cotton, peanut and Johnsongrass.

The mode of action of atrazine and simazine in
susceptible and resistant lines of corn, had been reported
by Eastin et al. (33). G.T.112 RfRf was used as resistant
and G.T.112 as the susceptible line. They found that
glucose and sucrose supplied to the treated plants through
a severed leaf tip protected the susceptible line from the
toxic effect of atrazine and simazine. The oxygen uptake
by excised roots of G.T.112 treated with atrazine gradually
declined with the age until the death of the plant. Atrazine
decreased root respiration in G.T.112 but had no effect in
G.T.112 RfRf. Root respiration in untreated G.T.112 was
higher than in untreated G.T.112 RfRf. Atrazine decreased
leaf respiration in both lines.

As previously indicated, no work has been done to
elucidate the effects of triazines on the nitrogen metabolism
of plants. However, much work has been reported about the
mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) in
plants. A review of the literature regarding the influence

of 2,4-D on certain aspects of nitrogen metabolism of plants, therefore, appeared desirable.

Protein and free amino acids: In many instances application of 2,4-D to plants affected protein and free amino acids metabolism. In general, 2,4-D increased protein and free amino acids in the stem and decreased these constituents in the leaves and roots.

The increase in protein content by 2,4-D has been reported by Sell et al. (94) in bean stem, by West et al. (119) in cucumber stem, by Basler and Nakazawa (10) in cotyledonous leaf tissue and by Dunham (32) in wheat and barley grains. The increases in protein content also were found by Freiberg and Clark (40) in the root and stem of soybean and by Payne et al. (82) in potato tubers. Sell et al. (94) showed that 2,4-D caused an increase in protein content due to the depletion of carbohydrate.

Sell et al. (94) demonstrated that 2,4-D increased arginine, histidine, isoleucine, leucine, phenylalanine, valine, lysine and methionine content in bean stem. Free amino acids were reduced by 2,4-D, according to Weller et al. (118), in roots and leaves of bean plant. One-half lb. of 2,4-D per acre decreased the amount of 11 amino acids and increased glutamic acid content in potato tubers (82). Free amino acids were increased in the tops of potato and sugar beet plants and were decreased in bean tops when 2,4-D was applied to the foliage (44).

Akers and Fang (1) exposed 2,4-D treated bean plants to $C^{14}O_2$. They found greater incorporation of $C^{14}O_2$ into the aspartic and glutamic acids, but the amount and percentage of these amino acids were considerably smaller in the treated plants.

Ribonucleic acid: The increase in RNA content of 2,4-D treated tissues has been reported by many investigators. This increase has been shown by Rebstock et al. (86) in bean plants, by West et al. (119) in cucumber stem, by Basler and Nakazawa (10) in cotyledonous leaf tissue of young cotton seedlings and by Key and Hanson (62) in soybean seedlings.

The location of the nucleic acid within the cell components was altered by 2,4-D, according to Basler and Nakazawa (10). They found more RNA in the soluble protein and less RNA in the particulate protein. West et al. (120) showed a marked increase in the RNA content in the microsomal portion of the cytoplasm of cucumber tissue. Chrispeels and Hanson (23) reported doubling of RNA in both microsomal and soluble fractions obtained from 2,4-D treated soybean hypocotyl.

According to Key and Hanson (61, 62), the incorporation of ADP-C^{14} in RNA of soybean hypocotyl was enchanced by 2,4-D application. Low concentration of 2,4-D was found to decrease RNA in cucumber tissue (119).

Chloroplast Structure

A chloroplast, the site of photosynthesis, acts
as energy capturing, storing and transferring device in
photosynthesis. The grana containing chloroplasts of
higher plants are lamellar. These lamallae are differen-
tiated into non-pigmented stroma and the pigmented
chlorophyll containing grana. The ultra-micro structure
consists of lamellae. In the absence of chlorophyll,
the lamellae are not formed (123). The photosynthetic
pigment, the chlorophyll and carotenoids, are concen-
trated within the dense lamellae of the grana containing
chloroplasts and in the lamellar chloroplasts (103).

Frey-Wyssling (41) proposed that a chloroplast
consists of 20 to 30 parallel lipid layers. These layers
are separated from the aqueous protein layer by macro-
molecular films of chlorophyll molecules. The hydro-
phillic porphyrin "head" of each chlorophyll molecule
extends into the aqueous protein complex and the lipophillic
phytol "tail" reaches into the lipid layer (41). It was
further postulated that each fatty layer is 50Å thick
and is separated from another by aqueous protein. As
yet, no chemical analyses of these layers has been possible.
The location of these layers has been assumed from the
chemical reactions of the fixing agents and various strains
with biological materials (107).

The concentrations of chlorophyll within the
chloroplast vary in a variety of plant chloroplasts (127).
Trurnit (107) suggested that the chlorophyll molecule lies
at an angle of 35° to 55° within the lamellae.

The precise composition and chemistry of the
active chloroplast is still unknown. The gross analysis
of isolated chloroplasts indicates that they consist of
35 to 55 per cent protein, 18 to 37 per cent lipids--
mostly phospholipids--and 5 to 8 per cent inorganic
materials on dry weight basis (126). The composition of
lipid is not uniform in various plants, although galacto-
lipids are present and linolenic acid predominates (11, 27,
122). Nucleic acids - RNA and DNA - have been identified,
with estimates from 0.3 to 3 per cent on dry weight basis
(15, 83). The chloroplast pigment - the chlorophyll
averages about 6 per cent and carotenoids 2 per cent on
dry weight basis. The mean total phosphorus is 0.4 per
cent on dry weight basis. This phosphorus includes
inorganic phosphorus, phosphatides and nucleic acid
phosphorus (124).

Cytochrome-f and cytochrome-b_3 have been found
in the chloroplasts by Hill (55). These two cytochromes
together could make up as much as 20 per cent of total
protein content of the chloroplasts (55). Small amounts
of carbohydrate, mostly arabinose and galactose, have
been found in chloroplasts (67).

In Euglena, the chloroplast chlorophylls
constitute about 85 per cent chlorophyll a and 15 per cent
chlorophyll b (125). The major carotenoids are β-carotene,
lutein and neoxanthin. However, in Euglena, instead of
lutein, 80 per cent of the carotenoids present were

identified as antherxanthin, 11 per cent β-carotene and 7 per cent neoxanthin (63).

Synthesis of the polypeptide bond in chloroplast has been studied by Sissakin (98) using C^{14} and S^{35} isotopes. He concluded that peptide bond synthesis can be independently carried out by the chloroplasts as well as the mitochondria.

Protein Synthesis

Protein synthesis requires: 20 different L-amino acids, 20 different amino acid-activating enzymes, at least 20 separate kinds of soluble RNA (sRNA), energy source, divalent ions, a complex aligning mechanism containing some sort of genetically controlled code, and various additional enzymes for peptide bond formation, release of the complex protein from the "template" and reactivation of the sRNA.

Results of many experiments suggested that the mechanism of protein synthesis involves the following chemical steps:

Step I- Amino acid activation

Amino acid + sRNA + ATP $\xleftrightarrow{Mg^{++}}$ Amino acid-sRNA + AMP + P-P
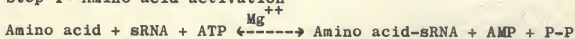
Step II- Polypeptide formation

n(Amino acid-sRNA) + Ribosome $\xrightarrow{GTP, Mn^{++}}$ Polypeptide attached to ribosome + nsRNA
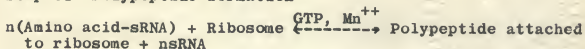
Step III- Release of polypeptide from ribosome

Polypeptide attached to ribosome $\xrightarrow{ATP, Mg^{++}}$ Polypeptide + Ribosome
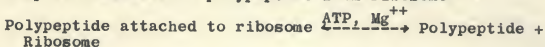
Step IV- Formation of secondary and tertiary structure

Amino acid activation: It is now well established that the enzyme responsible for the activation of amino acids occurs in the cells of many different kinds of plants, animals and microorganisms (24, 29, 30, 78). This enzyme is specific and activates only L-amino acids and some analogues (24, 65, 76, 77, 114). These enzymes have been purified from various sources.

The one end of amino acid attaches with enzyme and the carboxyl end esterifies with ADP. This linkage of carboxyl group to AMP has been supported by pyrophosphate exchange reactions (30, 31, 57), by the synthesis of ATP from pyrophosphate and synthetic amino acid-AMP (31, 64) and by the transfer of O^{18}_2 from the amino acid carboxyl to the AMP phosphate (12, 59).

Soluble ribonucleic acid: The most obvious mechanism for specifying the order of amino acids in a forming polypeptide chain is by the sequential base-pairing of each sRNA molecule (with attached amino acid) with a particular sequence of bases in ribosomal RNA.

The X-ray diffraction pattern of total adapter or sRNA resembles the diffraction pattern of DNA (17). The observed orientation strongly confirms the double-helical nature of sRNA. Recently, Spencer et al. (99) succeeded in crystallizing a sRNA from yeast, which they believe to be adapter RNA. On the basis of X-ray diffraction studies, they proposed that adapter RNA from yeast has completely regular Watson-Crick type base pairing.

The sedimentation constant and molecular weight of sRNA from Escherichia coli has been reported by various workers from 2.50 to 4.65S and 15,000 to 50,000 respectively (15, 104). The molecular weight of about 24,000 indicates a chain length of about 67 nucleotides (17). This suggested that the structure consists of a single polynucleotide chain with a hair pin bend approximately in the middle.

All adapter RNA have the terminal sequence of amino acid-adenylic acid-cytidylic acid-cytidylic acid on one end and guanine on the other. The sequence of nucleotides in between the two terminal groups is not known. With the two free nucleotides on the amino acid end, the third nucleotide (cytidylic acid), could make a base pair of the Watson-Crick type with guanine from the other end of the adapter RNA. The three unpaired nucleotides in the bend serve as the coding trinucleotide sequence which is presumed to react by specific base pairing with messenger RNA (mRNA) template. The unpaired A-C grouping on the end, carrying the amino acid may give amino acids on adjacently adsorbed adapter RNA sufficient freedom of movement to make contact.

Polypeptide formation: The theory of how amino acids are ordered on ribosomes was proposed by Crick (26). The amino acids linked with specific adapter RNA are transferred to the ribosome where the adapter RNAS' are joined by hydrogen bonds to complementary sites on an RNA-template

called mRNA and the amino acids are placed in the proper
juxtaposition for polypeptide synthesis. The amino acids
are added sequentially to the N-terminal end of the
growing polypeptide chain on the surface of ribosome
(115, 128).

The transfer of amino acid-sRNA to the reti-
culocyte microsome requires GTP, ATP, an ATP-generating
system and an enzyme fraction (58). This enzyme was
found to consist of both a heat-stable and heat-labile
fraction (49). The heat-stable factor can be replaced
by glutathione (73) and increased concentration of ATP
and GTP (69). Similar requirements for the transfer
reactions have been noted with pea ribosomes (115).
Under such conditions, pea ribosomes will catalyze the
conversion of amino acid-sRNA into "soluble protein" at
considerably greater rates than are obtained with an
equivalent quantity of free amino acids (116). This
finding strongly supports the idea that sRNA-bound amino
acids are intermediates in protein synthesis. The enzyme
responsible for the transfer of amino acids from sRNA
to ribosome-bound polypeptide has been partially purified
(13, 69, 73, 117).

Messenger ribonucleic acid: The template for protein
synthesis called mRNA, is separable from the ribosome (115).
Zubay (131) assumed that mRNA, when active, exists in the
form of a single polynucleotide chain with no intra-or
intermolecular hydrogen bonding.

Zamecnik (129) proposed a template structure. In his model, the bases of two molecules interact with the polypeptide chain in the completely extended configuration producing a hydrogen-bonded, stepladder-like structure analogous to the extended beta-configuration in protein. This is accompanied by orienting the polynucleotide chain of the mRNA in such a way that all the bases are situated on one side, and phosphate groups on the other. The three alleged coding nucleotides in the bend of the adapter RNA adopt a similar extended configuration. The translation distance per three nucleotides for extended mRNA would be 20 to 40 $\overset{o}{A}$, which is very nearly the distance of close approach of adjacent adapter RNA double helices. Amino acids on adjacent adapter molecules could not interact unless provided with flexible extension. The postulated A-C terminal grouping of the RNA provides the minimum number of unpaired nucleotides to give the necessary reach.

The sequence of nucleotides in the synthetic mRNA had been related to the sequence of amino acids in the product polypeptide chain. Messenger RNA is read starting from the end with an unesterified 3'- hydroxyl group (110).

Ribosomes: The general structure and amino acid composition of ribosomes from animals, plants and micro-organisms are the same (20, 87, 108). Ribosomes are spherical structures with a diameter of 200-300 $\overset{o}{A}$. The

spherical ribosomes generally observed in extracts of
E.coli, have a sedimentation constant of 70S and mole-
cular weight of 2,600,000 (106). The 70S ribosome occurs
in the presence of approximately $10^{-3}\underline{M}$ magnesium ion.
At higher concentrations two 70S particles dimerize to
form one 100S particle. The 70S ribosomes from E.coli
are composed of two ribonucleoprotein sub-units, 30S
and 50S, with a molecular weight of $0.9X10^6$ and $1.5X10^6$,
respectively (106). Each sub-unit is constituted by
dry weight of about two-thirds RNA and one-third protein.

Both 50S and 30S particles are required in
protein-stimulated polypeptide synthesis in vitro
(68, 105). Which of the larger particles are active in
protein synthesis is not known (88, 105), but at present
it seems likely that 70S ribosome is the smallest
potentially effective unit. A significant amount of
ribosomal RNA may be located inside the 70S ribosome (97).

Zubay (131) described some of the important
properties of ribosomes.

1. Ribosomes in the process of protein synthesis do
not dissociate as readily into their respective 50S and
30S sub-units as do these ribosome which are inactive
(68, 105).

2. mRNA is dissociated from the ribosome and ribosomes
may associate more than one mRNA with varying molecular
weight (74, 75).

3. The life time of the ribosome in bacterial cells
is much greater than that of the mRNA (60).

4. The 30S sub-unit and the 70S particle have been
shown to form a complex with synthetic mRNA under
conditions where the 50S sub-unit does not (80).

5. The 30S sub-unit in E.coli contains a latent
ribonuclease.

The size of ribosomes must accomodate mRAS'
of various sizes. In most cases, mRNA molecule would
be too large for the entire molecule to be in direct
contact with the ribosome simultaneously. Probably
one part of mRNA is bound at one time. Adjacently
adsorbed adapters might, for greater stabilization,
be adsorbed to the outer surface of the 50S particle,
and the polypeptide synthesis thus would be more closely
associated with 50S ribosome. Zubay (131) gave the
function of ribosomes in his model as to provide a
"stabilizing surface" for maintaining the mRNA in a
configuration that would be thermodynamically unstable
in a solution and for maintaining the reacting adapter
molecules in parallel register.

Considering the size of ribosomes and the
length of mRNA, the idea of polyribosome or polysome
emerged recently. Polyribosomes are clusters of ribosomes
held together by RNA. Polysomes were first characterized
in the rabbit reticulocyte (112). According to this
idea, polysomes rather than the single ribosomes are
active units for protein synthesis (46, 112, 113).

Release of completed polypeptide from ribosome:
The existence of a separate reaction for the release of
a completed polypeptide chain from ribosomes is clearly
indicated by the observation that both pea ribosomes
(117) and reticulocyte ribosomes (13) will convert amino

acid bound to sRNA into "soluble protein" in the presence
of an unfractionated cell extract, but not in the presence
of the purified "transfer enzyme". Release of the
completed polypeptide chain is obtained only upon the
addition of a second protein. This release also requires
the presence of ATP and a divalent cation (53, 117).

MATERIALS AND METHODS

In this investigation, Seminole variety of
oats (<u>Avena</u> <u>sativa</u> L.) was the experimental material.
This plant is susceptible to simazine and has been
extensively used to assess the toxicity of simazine in
soil.

Planting in Sand Culture

Uniform seeds were selected and treated with
Arasan-75 and Captan-75 (1:1) fungicide. Oats seed
were planted in sand in 4-inch paper cups. Hoagland-
Arnon complete nutrient solution (56) was added each
day. Simazine at the rates of 0, 0.5 and 2.0 lbs.
active ingredient (AI) per acre was used pre-emergence
in these experiments. The calculated amount of
simazine was dispersed in nutrient solution and applied
to soil after planting.

The seeds were germinated and grown for 12
days in a growth chamber with controlled temperature,
photoperiod and light intensity. The day and night
temperatures were maintained at $72^{O}F$ and $65^{O}F$, respec-
tively. The photoperiod was adjusted to 12-hour day
and light intensity was maintained at 1,500 foot candle.
A different set of plants was grown for each experiment.

Fresh and Dry Weights

Four plant samples per simazine treatment of 0, 0.5 and 2.0 lbs. AI per acre were collected at two-day intervals starting six days after treatment. Each sample consisted of all plant parts above the soil surface and was washed with deionized water immediately after harvest and blotted to dryness. The samples were dried for 24 hours in an oven at 85°C. After cooling of the samples, dry weights were determined. Fresh and dry weights were calculated on a per plant basis.

Total Protein

The total protein content of shoot tissue of plants grown in sand treated with simazine at 0, 0.5 and 2.0 lbs. AI per acre was determined in four replications. Samples were collected at two-day intervals, starting six days after treatment.

The plants were harvested, washed, dried and cut into smaller pieces. These pieces included in a given sample were transferred into a scored beaker to which deionized water was added to a total volume of 70 ml. The tissue was homogenized in an omnimixer at 0°C. The homogenate was filtered through a four-layer cheese cloth and centrifuged at 400Xg to remove cell debris. The decanted supernatant was used for protein determination.

The protein was precipitated by 10 per cent cold trichloroacetic acid (TCA). The chlorophyll and lipid were removed from the pellet by washing several times with undiluted acetone. The protein pellet was dissolved in 0.01M sodium hydroxide (NaOH). The quantity of protein in the tissue was determined by the method of Lowry et al. (66). The standard curve for protein estimation was prepared for bovine serum albumin (BSA).

To the protein aliquot, 5 ml of 4 per cent sodium carbonate, 4 per cent sodium tartrate and 2 per cent copper sulfate mixture were added. After one hour, phenol reagent was added and mixed thoroughly. The volume was made to 10 ml and after 30 minutes the optical density of the sample was read on Beckman DB spectrophotometer at 750 mμ wavelength.

Total Ribonucleic Acid

Total RNA content of plants grown in sand treated with 0, 0.5 and 2.0 lbs. simazine per acre was determined by the method of Ogur and Rosen (79). The experiment was replicated four times. Samples were collected at two-day intervals starting six days after treatment.

The procedure for the collection of samples and homogenization was similar to that used for protein determination. The supernatant after removal of cell debris was used for the precipitation of total RNA.

The RNA was precipitated by 1N perchloric
acid at 0°C. The chlorophyll and lipid were removed
from RNA pellets by several washings with a mixture
of cold ethanol, ether and chloroform (2:2:1 V/V).
The RNA in the pellet was hydrolyzed in 0.5M NaOH.
After complete hydrolysis of RNA, the pH of the
hydrolyzate was adjusted to 7.0 and 2.0. Protein
precipitated at these pH levels and was removed by
centrifugation. The absorption spectrum of the samples
was from 320 to 220 mμ wavelength, respectively. The
total RNA was calculated by the following equation:

$$\mu g \text{ of RNA} = \text{O.D. at } 260\text{-O.D. at } 290 \text{ m}\mu \times 57$$
$$\times \text{ dilution} \times \text{volume of RNA solution}$$

Chlorophyll

Total chlorophyll, chlorophyll a and chloro-
phyll b of plants grown in sand treated with 0 and 2.0
lbs. of simazine per acre was determined by the method
of the Association of Official Agricultural Chemists (9).
In all, four samples in three replications were collected
at two-day intervals starting six days after treatment.
The seedlings were harvested and weighed. Tissue was
chopped into smaller pieces and mixed with 0.1 gram of
calcium carbonate. The tissue was homogenized in 85 per
cent acetone. The homogenate was filtered and the residue
was washed several times with 85 per cent acetone to
remove chlorophyll. An aliquot of 20 ml was transferred

to a separatory funnel containing ether. In this way,
all the acetone was removed from the extract. The
ether extract was washed several times with water.
This chlorophyll extract was dried on anhydrous sodium
sulfate. The dried extract was diluted so that the
absorbance at 652 mμ did not exceed 0.8. The absorbance
of chlorophyll extract was read on spectrophotometer at
652 and 634.5 mμ with 0.05 mm slit width. Total
chlorophyll, chlorophyll a and chlorophyll b were
calculated by the following equation:

Total chlorophyll (mg per liter) = 7.12 A652 + 16.8 A634.5

Chlorophyll a (mg per liter)　　= 9.93 A652 - 0.777 A634.5

Chlorophyll b (mg per liter)　　= 17.6 A634.5 - 2.81 A652

Where A = Absorbance

Free Amino Acids

Triplicate samples of 1.5 grams fresh tissue
from sand treatments of 0 and 2.0 lbs. of simazine per
acre were collected at two-day intervals starting six
days after treatment. The tissue was homogenized in
80 per cent hot ethanol in an omnimixer. The homogenate
was centrifuged at 800Xg. The sediment was washed with
ethanol and centrifuged. The supernatants were pooled
and concentrated by passing a stream of air through them.
The volume was made up to 3 ml, centrifuged and the
supernatant used for chromatography.

A measured volume of amino acid standard and
of sample was chromatographed on Whatman number 1 filter

paper. The amino acids were separated by two-
dimensional, ascending paper chromatography. The
solvents used were n-butanol, acetic acid and water
(25:6:25 V/V) and phenol and water (10:2 V/V). Color
was developed by 0.3 per cent ninhydrin in 95 per cent
ethanol sprays. The Rf value for each spot was calculated.
The amino acids in the sample were identified with the
help of Rf values of standards.

A standard curve for L-leucine was prepared.
For this, a different amount of leucine was chromato-
graphed separately on Whatman number 1 filter paper.
After drying of these spots, color was developed by
ninhydrin spray. These spots were cut and eluted in
3 ml of 80 per cent ethanol and the optical density at
570 m μ wavelength was read on spectrophotometer. The
standard curve was prepared by plotting concentration
of L-leucine on X-axis and optical density on Y-axis.

The identified spots of the free amino acids
of sample were cut and eluted in 80 per cent ethanol.
The optical density of these spots was recorded. The
concentration of each amino acid present in the sample
was calculated from standard curve and expressed as
L-leucine per gram fresh tissue.

Particulate Proteins

Triplicate samples of plants from treatments
of 0 and 2.0 lbs. of simazine per acre were collected
at two-day intervals starting six days after treatment.

The weighed and washed tissue was homogenized in $0.5\underline{M}$ glucose, $0.02\underline{M}$ magnesium chloride and $0.02\underline{M}$ phosphate buffer at pH 7.0.

The homogenate was filtered through a four-layer cheese cloth. The filterate was centrifuged at 2,000Xg for 10 minutes to sediment chloroplasts. The supernatant fraction was centrifuged at 15,000Xg for 25 minutes to sediment mitochondria. Microsomes were sedimented by centrifugation of the supernatant at 105,000Xg for two hours in ultra-centrifuge. The supernatant fraction obtained after isolation of micro-somes was used for the estimation of soluble protein. Each sediment was suspended in the homogenization mixture.

The protein in each fraction was precipitated by 5 per cent cold TCA. The chlorophyll and lipids were removed by washing the protein pellet several times with undiluted acetone. The protein was dissolved in $0.01\underline{M}$ NaOH and determined by the method of Lowry et al. (66).

Radioisotope Experiments

Incorporation of C^{14}-leucine in protein: The radioactive leucine was obtained from Schwarz BioResearch Inc. with more than 99.5 per cent purity. The isotope was dissolved in $0.01\underline{N}$ HCl and its specific activity was 20.1 millicounts per mM.

Incorporation of C^{14}-leucine in total and chloroplast proteins of check and simazine treated plant tissue

Duplicate samples of plants from treatments of 0 and 2.0 lbs. of simazine per acre were collected six and ten days after treatment. The weighed tissue was chopped into one centimeter sections. These sections were incubated at $23^{O}C$ for 1.5 hours in 0.002$\underline{M}$ potassium citrate, 0.001$\underline{M}$ ammonium biphosphate, 0.003$\underline{M}$ sucrose and 5 μc C^{14}-leucine. After incubation, the tissue was washed several times with water and leucine to remove any absorbed free C^{14}-leucine. The tissue was homogenized in 10 per cent sucrose, $5X10^{-3}\underline{M}$ Tris and $10^{-4}\underline{M}$ $MgCl_2$ at $0^{O}C$.

The homogenate was filtered through a four-layer cheese cloth. This filterate was used for the precipitation of total protein. The chloroplasts in the cleared homogenate were sedimented at 2,000Xg for 10 minutes at $0^{O}C$. The chloroplasts were suspended in the homogenization media.

The protein was precipitated by 1$\underline{M}$ perchloric acid. The protein pellets were washed several times with undiluted acetone to remove chlorophyll and lipid. These pellets were washed twice with $1.5X10^{-5}\underline{M}$ leucine in 0.2$\underline{M}$ perchloric acid to remove radioactivity bound to the protein other than peptide linkage. The protein was dissolved in 0.01$\underline{M}$ NaOH and this solution was transferred to planchets. Planchets were dried and the radioactivity was counted with a Nuclear Chicago gas flow counter, using Q-gas at 4 lbs. pressure per square

inch. The number of counts per minute per gram fresh
tissue was calculated.

Incorporation of C^{14}-leucine in total and chloroplast protein of tissues incubated with and without simazine

The procedure followed in this experiment was the
same as that followed in the previous experiment except
simazine at 1 p.p.m. concentration was added in the
incubation mixture. The incubation period was increased
to three hours and the quantity of C^{14}-leucine added in
the incubation mixture was reduced to 3 µc.

Incorporation of C^{14}-leucine in the total and chloroplast proteins of 10-day old check and simazine treated plant tissue incubated with and without glucose solution

The procedure used in this experiment was the
same as has been described earlier. Glucose at the
concentration of 6 per cent was added in the incubation
mixture. The incubation time was three hours and the
amount of radioactive leucine used in incubation was
1.5 µc.

Incorporation of C^{14}-leucine in the total protein of 10-day old check and simazine treated tissue grown in light and dark

In this experiment, duplicate samples of fresh
tissues were collected from the following treatments:

1. 0 lbs. of simazine per acre and plants grown in
    light

2. 0 lbs. of simazine per acre and plants grown in
    dark

3. 2.0 lbs. of simazine per acre and plants grown in light

4. 2.0 lbs. of simazine per acre and plants grown in dark

The procedure for sample preparation, protein precipitation and radioactivity counting was the same as that mentioned earlier.

Incorporation of P^{32} in ribosomal RNA: Oat seedlings for this experiment were grown in water culture using Hoagland-Arnon complete nutrient solution. These seedlings were grown in a room with constant temperature and light. The calculated amount of simazine was added in the treated containers. The seedlings were grown for nine days. On the ninth day, the nutrient solution of the containers was replaced by the nutrient solution containing P^{32}. On the tenth day, shoots were harvested and used for determination.

Determination

The weighed and washed tissue was homogenized in a mixture of 5 per cent sucrose, $5 \times 10^{-3}\underline{M}$ Tris and $10^{-3}\underline{M}$ $MgCl_2$. The homogenate was filtered through a four-layer cheese cloth. The filterate was centrifuged at 15,000Xg for 20 minutes to remove the cell walls, chloroplasts and mitochondria. The volume of the supernatant fraction was reduced to 10 ml by lypholyzation. This extract was made at a concentration of 0.5 per cent deoxycholate (DOC).

A density gradient from 30 per cent to 5 per
cent sucrose was prepared. The extract was added on
the top of the density gradient and was centrifuged
in a swinging bucket, number 25.1, at 23,000 r.p.m.
in ultra-centrifuge for two hours. Two ml fractions
were collected in which RNA was precipitated by 1$\underline{M}$
perchloric acid and defatted by ethanol, ether and
chloroform mixture. The RNA was hydrolyzed in 0.5$\underline{N}$
NaOH. The pH of the hydrolyzate was adjusted to 7.0
and the absorption at 260 and 290 mμ was read on
spectrophotometer. The radioactivity in these fractions
was counted with a Nuclear Chicago gas flow counter,
using Q-gas at 4 lbs. pressure per square inch.

STATISTICAL ANALYSIS

The data from different experiments were
analyzed in factorial randomized design as described
by Steel and Torrie (102).

EXPERIMENTAL RESULTS

The present investigation was conducted to
gain a better understanding of the influence of simazine
on the physiology of plants. As mentioned earlier,
the Seminole variety of oats was the experimental
material in which the influence of this herbicide on
nitrogen metabolism was studied in detail.

Toxicity Symptoms

The seed planted in sand and treated with
simazine germinated and the seedlings emerged normally
on the fourth day. The development of phytotoxicity
symptoms are shown in Figures 1, 2, 3, 4 and 5. No
distinct symptoms of phytotoxicity appeared before
the yellowing of the entire leaf blade six days after
treatment. The leaf tips became white about eight days
after treatment and the leaves started drying two days
later. The plants become completely dessicated 14 days
after simazine application.

Fresh and Dry Weights

As indicated by the data in Table 1, the rate
of 0.5 lbs. of simazine slightly increased fresh weight
of plants in the sample analyzed six days after treat-
ment. In later samples, the fresh weight of plants
from this treatment decreased. This decrease was as

Fig. 1. Influence of different rates of simazine on the growth of oat plants, five days after treatment (0, 1/2 and 2 indicate 0, 0.5 and 2.0 lbs. simazine per acre, respectively).

Fig. 2.   Influence of different rates of simazine on
the growth of oat plants, six days after
treatment (0, 1/2 and 2 indicate 0, 0.5 and
2.0 lbs. simazine per acre, respectively).

Fig. 3. Influence of different rates of simazine on
the growth of oat plants, eight days after
treatment (0, 1/2 and 2 indicate 0, 0.5 and
2.0 lbs. simazine per acre, respectively).
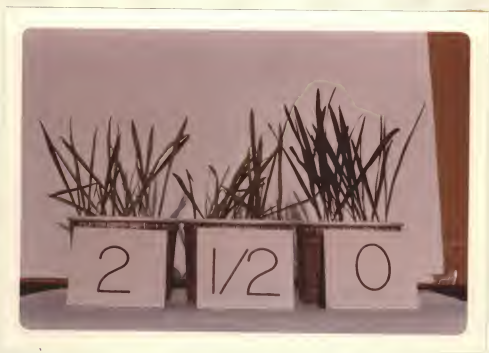
Fig. 4. Influence of different rates of simazine on
the growth of oat plants, 10 days after
treatment (0, 1/2 and 2 indicate 0, 0.5 and
2.0 lbs. simazine per acre, respectively).
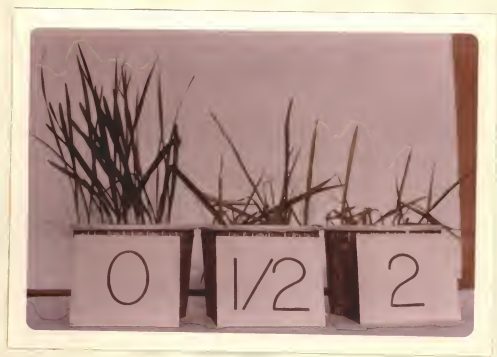
Fig. 5. Influence of different rates of simazine on
the growth of oat plants, 13 days after
treatment (0, 1/2 and 2 indicate 0, 0.5 and
2.0 lbs. simazine per acre, respectively).

TABLE 1

A COMPARISON OF THE CHANGES OF FRESH AND DRY WEIGHTS
OF OAT PLANTS TREATED WITH SIMAZINE

Days after planting and simazine application	Rate of simazine (lbs./A)	Fresh and dry weights (mgm/p)[1]	
		Fresh Weight	Dry Weight
6	0.0	109.50	9.08
	0.5	117.80	9.09
	2.0	108.20	8.33
8	0.0	139.00	13.57
	0.5	104.60	8.75
	2.0	99.30	8.27
10	0.0	177.69	16.49
	0.5	105.75	9.66
	2.0	107.93	9.28
12	0.0	223.24	20.64
	0.5	71.90	8.91
	2.0	102.50	10.72

[1]Each datum comprises the mean of four determinations.

much as 67 per cent in the sample collected 12 days
after simazine application.

No marked differences in the fresh weight
were found in the 2.0 lbs. per acre treatment when the
samples were collected six days after treatment. How-
ever, the fresh weight of plants from this treatment
decreased in later samples. The maximum decrease to
the extent of 54 per cent in the fresh weight of this
treatment was found in the last sample. The fresh
weight of 0 lb. treatment increased in the subsequent
samples, but the fresh weight of 2.0 lbs. treatment
was about the same throughout the growth period.

Table 1 shows that the rate of change in dry
weight due to simazine treatment is approximately the
same as fresh weight. No significant differences were
found between the dry weight of plants from 0 and
0.5 lb. of simazine treatments in the sample collected
six days after treatment. Differences in the dry weight
due to simazine treatment occurred in the samples
collected 8, 10 and 12 days after simazine application.

The statistical analysis of fresh and dry
weight (Table 3) shows highly significant differences
among treatments, sampling intervals and interaction.

Total Protein

The changes in protein concentration of oat
shoots as the result of simazine treatment are shown in
Table 2. The rate of change was the same on both fresh

TABLE 2

CHANGES IN TOTAL PROTEIN OF OAT SHOOTS
CAUSED BY SIMAZINE TREATMENTS

Days after planting and simazine application	Rate of simazine (lbs./A)	Protein content (mgm)[1]		
		/gr. fr. wt.	/gr. dry wt.	/plant
6	0.0	21.61	289.42	2.37
	0.5	20.35	263.74	2.40
	2.0	22.28	281.62	2.41
8	0.0	19.02	265.44	2.65
	0.5	18.05	194.76	1.89
	2.0	22.12	240.44	2.20
10	0.0	14.25	153.47	2.53
	0.5	13.60	145.78	1.44
	2.0	12.50	140.38	1.35
12	0.0	10.50	113.61	2.34
	0.5	9.85	86.23	0.71
	2.0	9.02	79.49	0.92

[1] Each datum comprises the mean of four determinations.

TABLE 3

ANALYSIS OF VARIANCE OF FOUR SIMAZINE EFFECTS ON OATS

Sources of variation	D.F.	Variance			
		Fresh weight (mgm/p)	Dry weight (mgm/p)	Protein (mgm/gr. dry wt.)	RNA (mgm/gr. dry wt.)
Treatments	2	19350.39**	180.59**	2653.15**	227.42**
Intervals	3	1380.30**	45.74**	125664.36**	6633.47**
Treatments X Intervals	6	4975.46**	27.47**	552.56**	152.53**
Error	36	3.11	0.09	170.74	2.41

* $P < .05$

** $P < .01$

and dry weight basis. The only observed difference in
these two results was that the protein content per gram
fresh tissue in the samples collected six and eight
days after simazine application was more in the plants
from 2.0 lbs. treatment than 0 lb. rate. The protein
content decreased in the shoots receiving the 0.5 lb.
treatment in all samples analyzed 6, 8, 10 and 12 days
after treatment. The protein concentration in the last
two samples (2.0 lbs. treatment) decreased more than
0.5 lb. treatment.

        The differences in protein contents of plants
in different treatments, sampling intervals and inter-
action were statistically significant at 1 per cent
probability level (Table 3).

Total Ribonucleic Acid

        Table 4 indicates an increased RNA content in
fresh tissue of plants from 2.0 lbs. simazine treatment
in the samples analyzed six and eight days after simazine
application. In the samples analyzed 10 and 12 days
after treatment, the RNA concentration in this treat-
ment decreased 40 and 70 per cent, respectively. The
0.5 lb. treatment decreased RNA content in all four
samples. However, in the last two samples RNA was more
affected by 2.0 lbs. treatment than 0.5 lb. The differ-
ences in the results calculated on dry weight basis
and fresh weight basis were that the concentration of
RNA per gram dry tissue in 2.0 lbs. treatment decreased

TABLE 4

CHANGES IN TOTAL RNA CONTENT OF OAT SHOOTS
CAUSED BY SIMAZINE TREATMENTS

Days after planting and simazine application	Rate of simazine (lbs./A)	RNA content (mgm)[1]		
		/gr. fr. wt.	/gr. dry wt.	/plant
6	0.0	4.50	54.27	0.49
	0.5	4.10	52.13	0.48
	2.0	4.82	54.28	0.52
8	0.0	3.79	53.04	0.53
	0.5	3.77	45.05	0.39
	2.0	4.42	52.18	0.44
10	0.0	2.87	35.91	0.51
	0.5	2.50	27.35	0.26
	2.0	2.40	24.85	0.26
12	0.0	1.53	19.55	0.34
	0.5	1.31	10.57	0.09
	2.0	0.96	7.17	0.10

[1] Each datum comprises the mean of four determinations.

in first and second samples also.

The per plant RNA content of plants treated with 2.0 lbs. simazine was slightly increased in the first sample. A marked decrease in RNA content occurred in both levels of simazine in the samples analyzed 8, 10 and 12 days after the application of simazine. The differences in the RNA content of treatments, sampling intervals and interaction were highly significant (Table 3).

Chlorophylls

The results of chlorophyll determination in the plants grown in sand treated with 0 and 2.0 lbs. of simazine per acre are listed in Table 5. In general, a rise occurred in total chlorophyll and chlorophyll a contents per gram fresh tissue in plants growing in the 0 rate of simazine application up to eight days of the growth period. These chlorophylls decreased in later samples. Chlorophyll b increased throughout the growth period. On the other hand, gradual increase in per plant total chlorophyll, chlorophyll a and chlorophyll b contents occurred in the subsequent samples.

These results clearly indicate that all chlorophylls had been affected by simazine treatment. In the earlier samples, chlorophyll b was not affected to the same extent as chlorophyll a. However, in the last sample, chlorophyll a and chlorophyll b per gram fresh tissue decreased as much as 67 and 74 per cent,

TABLE 5

CHANGES IN CHLOROPHYLL CONTENT OF OAT SHOOTS
CAUSED BY SIMAZINE TREATMENTS

Days after planting and simazine application	Rate of simazine (lbs./A)	Chlorophyll (mgm/gr. fr. wt.)[1]			Chlorophyll (mgm/p)[1]		
		Total Chlorophyll	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a	Chlorophyll b
6	0.0	16.46	12.67	3.80	1.56	1.20	0.36
	2.0	10.13	7.81	2.33	0.92	0.71	0.21
8	0.0	21.31	16.18	5.13	3.15	2.40	0.75
	2.0	9.54	7.01	2.52	1.13	0.82	0.31
10	0.0	20.84	15.60	5.24	4.21	2.95	1.26
	2.0	8.97	6.42	2.55	1.18	0.84	0.33
12	0.0	20.04	13.43	6.62	5.19	3.45	1.75
	2.0	6.10	4.42	1.67	0.58	0.41	0.16

[1]Each datum comprises the mean of three determinations.

respectively. This determination shows that the decrease in chlorophyll _b_ was greater than in chlorophyll _a_ in the sample collected 12 days after treatment.

The statistical analysis of these data shows that the differences in chlorophyll _a_ and chlorophyll _b_ due to simazine treatment are highly significant (Table 6). The variations among the sampling intervals, chlorophylls and various interactions except one were found significant at 1 per cent probability level.

Alcohol-Soluble Amino Acids

Free amino acids in the shoots of plants grown in sand treated with 0 and 2.0 lbs. of simazine per acre 6, 8, 10 and 12 days after treatment are shown in Table 7. The concentration of each amino acid is given in terms of L-leucine.

In general, the concentrations of free amino acids six and eight days after simazine application were higher in plants from 0 lb. than 2.0 lbs. simazine treatment. An opposite result was found in the last two samples where the concentrations of free amino acids increased in the plants subjected to the 2.0 lbs. treatment.

More specifically, in the first sample the concentrations of aspartic acid, serine, threonine, alanine and histidine decreased and the concentration of glutamic acid increased in the plants from 2.0 lbs. simazine treatment. No differences in lysine, phenyl-alanine and leucine contents were found between 0 and

TABLE 6

ANALYSIS OF VARIANCE OF CHLOROPHYLL
CONTENT OF OATS

Sources of variation	D.F.	Variance
		Chlorophyll (mgm/l/p)
Treatments	1	20.01^{**}
Intervals	3	1.63^{**}
Chlorophylls	1	10.97^{**}
Tr. X Intervals	3	2.12^{**}
Tr. X Chlorophylls	1	3.17^{**}
Intervals X Chlorophylls	3	0.12^{*}
Tr. X Intervals X Chlorophylls	3	0.17^{**}
Error	32	0.03

* $P < .05$

** $P < .01$

TABLE 7

A COMPARISON OF CHANGES OF ALCOHOL-SOLUBLE AMINO ACIDS
OF OAT PLANTS FROM SAND TREATED WITH SIMAZINE

Days after planting and simazine application	Rate of simazine (lbs./A)	m mole of L-leucine/gr. fr. wt.[1]								
		Aspartic acid	Glutamic acid	Serine	Lysine	Threonine	Alanine	Histidine	Phenyl-alanine	Leucine
6	0.0	0.061	0.101	0.105	0.068	0.076	0.082	0.078	0.063	0.041
	2.0	0.058	0.130	0.053	0.068	0.028	0.052	0.046	0.064	0.043
8	0.0	0.019	0.073	0.032	0.011	0.015	0.039	0.064	0.078	0.034
	2.0	0.019	0.046	0.028	0.117	0.011	0.027	0.059	0.073	0.036
10	0.0	0.017	0.019	0.054	0.124	0.009	0.015	0.029	0.068	0.037
	2.0	0.017	0.024	0.051	0.153	0.010	0.024	0.033	0.081	0.041
12	0.0	0.010	0.020	0.055	0.009	0.026	0.011	0.011	0.006	Trace
	2.0	0.028	0.015	0.057	0.125	0.011	0.011	0.029	0.029	0.032

[1] Each datum comprises the mean of three determinations.

2.0 lbs. treatment. The concentration of all the
mentioned amino acids, except lysine, decreased in
plants eight days after treatment with 2.0 lbs. of
simazine.

In the last two samples, concentrations of all
the reported free amino acids increased in plants from
the 2.0 lbs. treatment with this increase being most
pronounced in lysine.

The statistical analysis (Table 8) indicated
that the differences among the treatments, sampling
intervals, amino acids and interactions are highly
significant.

Particulate Proteins

The changes in particulate proteins of plants
grown in sand treated with 0 and 2.0 lbs. of simazine
per acre are shown in Table 9. Chloroplast proteins
were decreased 28 per cent by the sixth day after
receiving the 2.0 lbs. treatment. In contrast to
chloroplast proteins in this sample, the concentrations
of mitochondrial, ribosomal and cytoplasmic proteins
were increased by simazine treatment.

The chloroplast proteins of plants from 2.0 lbs.
of simazine decreased to about half in the sample
collected eight days after treatment. Simazine also
caused increased concentrations of mitochondrial and
ribosomal proteins.

TABLE 8

ANALYSIS OF VARIANCE OF CONTENTS OF
FREE AMINO ACIDS OF OATS

Sources of variation	D.F.	Variance Amino acids (m mole/gr. fr. wt.)
Treatments	1	0.0009**
Intervals	3	0.0106**
Amino acids	8	0.0061**
Tr. X Intervals	3	0.0023**
Tr. X Amino acids	8	0.0021**
Intervals X Amino acids	24	0.0020**
Tr. X Intervals X Amino acids	24	0.0005**
Error	72	0.000002

* $P < .05$

** $P < .01$

TABLE 9

PARTICULATE PROTEINS OF OAT SHOOTS
AS INFLUENCED BY SIMAZINE TREATMENTS

Days after planting and simazine application	Rate of simazine application (lbs./A)	Proteins (mgm/gr. fr. wt.)[1]			
		Chloroplast	Mitochondrial	Microsomal	Cytoplasmic
6	0.0	3.96	2.05	1.76	7.26
	2.0	2.85	2.25	2.15	7.65
8	0.0	4.69	1.29	1.50	7.25
	2.0	2.47	2.02	2.01	7.79
10	0.0	5.47	1.30	1.42	7.75
	2.0	2.19	1.08	1.06	5.17
12	0.0	4.88	1.22	1.02	7.73
	2.0	1.90	1.07	0.76	4.88

[1]Each datum comprises the mean of three determinations.

The simazine treatment decreased the concentrations of all the four proteins in the third and fourth samples collected 10 and 12 days after simazine application. In these samples too, chloroplast proteins were affected more than others. The maximum decrease of 61 per cent occurred in the chloroplast proteins in the last sample.

The data were analyzed statistically and the results are shown in Table 10. The differences among proteins due to treatments and sampling intervals as well as various interactions were highly significant.

Incorporation of C^{14}-Leucine in Protein

The results of C^{14}-leucine incorporation in the total and chloroplast proteins of plants from 0 and 2.0 lbs. simazine treatment are reported in Table 11. These incorporation studies were conducted to demonstrate the effect of simazine on the incorporation rate of C^{14}-leucine in protein.

In the first sample which was incubated six days after simazine application, incorporation of C^{14}-leucine in the total protein of tissues from the 2.0 lbs. simazine treatment was slightly enchanced. On the other hand, in the sample collected 10 days after simazine treatment, the rate of incorporation of radioactive leucine in the total protein of tissues grown in 2.0 lbs. treatment was reduced by three-fourths.

The results of this experiment show clearly that

TABLE 10

ANALYSIS OF VARIANCE OF CONTENTS OF
PARTICULATE PROTEINS OF OATS

Sources of variation	D.F.	Variance
		Protein (mgm/gr. fr. wt.)
Treatments	1	8.00^{**}
Intervals	3	6.94^{**}
Proteins	3	151.23^{**}
Tr. X Intervals	3	4.76^{**}
Tr. X Proteins	3	7.68^{**}
Intervals X Proteins	9	0.75^{**}
Tr. X Intervals X Proteins	9	1.27^{**}
Error	64	0.03

* $P < .05$

** $P < .01$

TABLE 11

RATE OF INCORPORATION OF C^{14}-LEUCINE IN TOTAL AND
CHLOROPLAST PROTEINS OF CHECK AND SIMAZINE
TREATED OAT SHOOTS[1]

Days after planting and simazine application	Rate of simazine (lbs./A)	Cpm./gr. fr. wt.[2]	
		Total protein	Chloroplast protein
6	0.0	6,901	2,515
	2.0	7,003	1,062
10	0.0	14,313	3,195
	2.0	3,695	840

[1]The tissue was incubated in 25 ml which contained 5 μc of C^{14}-leucine for 1.5 hours.

[2]Each datum comprises the mean of two determinations.

the incorporation rate of leucine in the chloroplast proteins of plants grown in 2.0 lbs. simazine treatment was decreased to less than half in the first sample. In the sample collected 10 days after treatment, the incorporation rate for chloroplast protein reduced by three-fourths, approximately the same rate of reduction for total proteins.

Another experiment was conducted to demonstrate again the effect of simazine on C^{14}-leucine incorporation in total and chloroplast proteins. In this experiment, instead of simazine application pre-emergence, plants were grown for 10 days without any previous treatment and the simazine at 1 p.p.m. concentration was added in the incubation mixture.

The results shown in Table 12 indicate that simazine decreased the incorporation rate of C^{14}-leucine in total and chloroplast proteins. The differences in incorporation rates in this experiment were not as distinct as in the previous experiment in which simazine was applied at time of planting. The results of both experiments, however, suggest that simazine inhibited the incorporation of C^{14}-leucine in protein.

Two other radioisotope experiments were conducted to determine if the incorporation rate of C^{14}-leucine in protein is affected by inhibition in photosynthesis or if it is independent of photosynthetic inhibition.

TABLE 12

RATE OF INCORPORATION OF C^{14}-LEUCINE IN
TOTAL AND CHLOROPLAST PROTEINS OF 10-DAY OLD
SHOOT TISSUE INCUBATED WITH AND WITHOUT SIMAZINE[1]

Treatments (p.p.m. simazine)	Cpm./gr. fr. wt.[2]	
	Total protein	Chloroplast proteins
0.0	10,525	1,896
1.0	9,640	1,740

[1]The tissue was incubated in 25 ml which contained
3 μc of C^{14}-leucine for three hours.

[2]Each datum comprises the mean of two determinations.

In the first experiment, 6 per cent glucose was supplied in the incubation mixture to the tissues grown in 0 and 2.0 lbs. simazine treatment. Table 13 shows that glucose inhibited C^{14}-leucine incorporation in total and chloroplast proteins of tissues from 0 lbs. treatment. In contrast, glucose increased C^{14}-leucine incorporation in total and chloroplast proteins of tissues grown in 2.0 lbs. simazine treatment. The incorporation rate was approximately doubled in the tissues from 2.0 lbs. treatment and supplied with glucose.

The results of C^{14}-leucine incorporation in proteins of tissues from 0 and 2.0 lbs. simazine treatment and grown in dark and light are shown in Table 14. The incorporation rate of C^{14}-leucine in the protein of tissue subjected to 2.0 lbs. simazine treatment and grown in light was slightly higher than for etiolated tissues of 0 lb. treatment.

Incorporation of P^{32} in Ribosomal RNA

The RNA contents of different sizes of ribosomes in the plants from 0 and 2.0 lbs. simazine treatment are listed in Table 15 and illustrated in Figure 6. The monosome $(70S)_1$, disomes $(70S)_2$, $(70S)_3$ and polysomes $7(70S)_3$ were found in the sixth, ninth, twelfth and pellet fractions of density gradient. In general, the concentrations of RNA in all the mentioned ribosomes were decreased by simazine treatment. These decreases were 33.8, 36.5, 36.4 and 39.8 per cent,

TABLE 13

RATE OF INCORPORATION OF C^{14}-LEUCINE IN TOTAL AND
CHLOROPLAST PROTEINS OF CHECK AND SIMAZINE TREATED
10-DAY OLD OAT SHOOTS INCUBATED WITH AND WITHOUT GLUCOSE[1]

Rate of simazine (lbs./A)	Per cent glucose in incubation mixture	Cpm./gr. fr. wt.[2]	
		Total protein	Chloroplast protein
0.0	6	5,554	1,554
	0	10,555	2,405
2.0	6	2,967	1,718
	0	1,310	786

[1]The tissue was incubated in 25 ml which contained
1.5 μc of C^{14}-leucine for three hours.

[2]Each datum comprises the mean of two determinations.

TABLE 14

RATE OF INCORPORATION OF C^{14}-LEUCINE IN TOTAL
PROTEIN OF CHECK AND SIMAZINE TREATED 10-DAY OLD
OAT SHOOT TISSUE GROWN IN LIGHT AND DARK[1]

Rate of simazine (lbs./A)	Cpm./gr. fr. wt.[2]	
	Light	Dark
0.0	672	285
2.0	385	214

[1]The tissue was incubated in 25 ml which contained
1 μc for 1.5 hours.

[2]Each datum comprises the mean of two determinations.

TABLE 15

INFLUENCE OF SIMAZINE ON RNA CONTENT AND RATE OF INCORPORATION OF P-32 IN RIBOSOMES OF OAT SHOOTS[1]

Fractions	Simazine at 0 lb./A²				Simazine at 2.0 lbs./A²				
	μg RNA/p	Cpm./p	Cpm./μg RNA	% of total Cpm.	μg RNA/p	% inhibition	Cpm./p	Cpm./μg RNA	% of total Cpm.
1	2.65	43.92	16.57	2.16	1.24	53.21	2.18	1.75	3.25
2	4.03	62.66	15.55	3.08	2.83	29.78	2.11	0.75	3.14
3	6.70	86.72	12.94	4.26	4.20	37.31	2.16	0.51	3.22
4	8.26	107.51	13.02	5.28	5.75	30.39	3.32	0.58	4.94
5	10.74	139.07	12.95	6.83	6.66	37.99	4.19	0.63	6.24
6	11.58	160.65	13.87	7.88	7.66	33.85	4.88	0.64	7.27
7	11.10	159.48	14.36	7.83	7.08	36.21	4.22	0.60	6.28
8	13.53	177.66	13.13	8.72	8.67	35.92	4.75	0.55	7.07
9	13.71	180.45	13.16	8.86	8.72	36.40	5.92	0.68	8.81
10	13.60	179.83	13.22	8.83	8.27	39.19	4.72	0.57	7.02
11	14.52	154.79	10.66	7.60	8.80	39.39	4.42	0.50	6.58
12	15.58	198.48	12.74	9.74	9.91	36.39	6.97	0.70	10.37
13	10.72	117.21	10.93	5.75	7.71	28.08	3.53	0.46	5.26
14	7.08	82.33	11.63	4.04	3.63	48.73	1.71	0.47	2.55
15	4.63	49.76	10.75	2.44	3.30	33.00	2.45	0.74	3.65
Pellet	11.23	137.04	12.20	6.73	6.76	39.80	9.64	1.43	14.35

[1] The roots of oat plants were immersed for one day in 500 ml nutrient solution which contained 50 μc of P³².

[2] Each datum comprises the mean of two determinations.

respectively in $(70S)_1$, $(70S)_2$, $(70S)_3$ and $7(70S)_3$ ribosomes.

The results of P^{32} incorporation in ribosomes are given in Table 15 and Figure 6. The incorporation rate of P^{32} in the RNA of ribosomes was almost completely blocked in the tissues from 2.0 lbs. simazine treatment. Only 3.3 per cent incorporation was found in the ribosomal RNA of plants subjected to 2.0 lbs. of simazine per acre treatment.

The ratios of different ribosomes with $(70S)_1$ ribosome in the tissues from 0 and 2.0 lbs. treatment are shown in Table 16. These ratios decreased slightly in tissues grown in 2.0 lbs. of simazine treatment. A greater decrease was found in the ratio of polysomes with monosomes.

Fig. 6.   Influence of simazine on the RNA content of
          different sizes of ribosomes and the rate of
          incorporation of P^{32} in the RNA of different
   sizes of ribosomes.
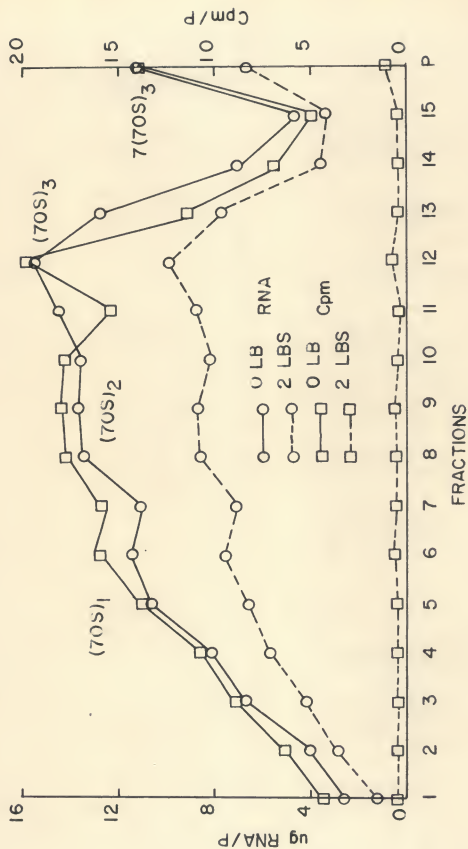
TABLE 16

A COMPARISON OF CHANGES IN THE RATIOS OF DIFFERENT
RIBOSOMES IN OAT PLANTS TREATED WITH SIMAZINE

Ribosomes	Ratios of ribosomes with monosome	
	0 lb. simazine/A	2.0 lbs. simazine/A
$(70S)_2/(70S)_1$	1.18	1.14
$(70S)_3/(70S)_1$	1.35	1.29
$7(70S)_3/(70S)_1$	0.97	0.88

DISCUSSION

In the studies reported here, the symptoms of simazine toxicity appeared first in the form of yellowing of leaves. Necrosis later began in the leaf tips and soon spread throughout. Death of the plants occurred 14 days after the application of simazine.

Similar development of phytotoxicity symptoms as a result of simazine application has been reported by Schneider (93) in wheat and Coleus blumei. Also, Rodgers and Wilcox (89) reported leaf chlorosis in oat plants seven days old grown in Lakeland fine sandy soil. These plants died 14 days after planting.

The fresh and dry weights of tissues from 0.5 lb. simazine treatment slightly increased in the first samples collected six days after treatment. However, fresh and dry weights of oat shoots decreased in later samples from this treatment. The fresh and dry weights of oat shoots from 2.0 lbs. simazine treatment were decreased in all samples. The maximum reduction in fresh and dry weights occurred in the last sample, collected 12 days after simazine treatment.

Burnside and Behrens (19) reported that the dry weight of oat plants was slightly increased by low concentrations of simazine and decreased by higher

71

concentrations. The results reported herein for higher concentrations of simazine are in agreement with these findings as well as with those of Moreland (71) and Sheets (95).

Photosynthesis is the process of green plants by which food reserves are synthesized. The net gain in fresh and dry weights depends on this fundamental process. Several workers (8, 34, 35, 42, 71, 91) have reported that triazines inhibit photosynthesis. Therefore, a determination of the relationship between this inhibition and the reduced fresh and dry weights of simazine treated tissues was logical. Other metabolic processes, however, may be inhibited by simazine and consequently influence fresh and dry weights.

A marked influence of simazine was observed on the chlorophyll contents of oat plants. In general, a reduction in total chlorophyll content of oat plants occurred. The maximum decrease of 67 per cent was found in the sample analyzed 12 days after simazine application. The analysis of chlorophyll a and chlorophyll b, which are the two major chlorophylls involved in photosynthesis, indicated that the concentration of both was decreased by this herbicide. In earlier samples, chlorophyll a was reduced more than chlorophyll b, but this finding was reversed in later samples.

The observations of Schneider (93) demonstrate qualitative changes in the chlorophyll contents of

simazine treated plants. Recently, Ashton and
Bisalputra (4) reported that atrazine caused a decrease
in the amount of chlorophyll present in Chlorella. No
published data are available regarding the quantitative
changes in chlorophylls as the result of simazine
application.

Since protein is an important component of
chloroplasts, a study of changes in protein contents
of oats was appropriate. In general, simazine inhibited
protein synthesis. Protein per gram fresh and dry
tissue of plants from 0.5 lb. simazine treatment decreased
in all samples. However, the protein content per plant
from this treatment slightly increased by the sixth day
after treatment. In other samples, protein per plant
was reduced. Protein per gram fresh tissue in the plants
subjected to 2.0 lbs. simazine treatment increased in
the first and second samples but decreased in samples
collected 10 and 12 days after treatment. The per plant
protein in the plants from 2.0 lbs. simazine treatment
slightly increased in the first sample, but decreased
in the later three samples. Protein per gram dry tissue
in the plants from 2.0 lbs. simazine treatment was
decreased in all samples.

In plants from the 2.0 lbs. simazine treatment,
the concentration of protein per plant was not decreased
in the first two samples. On the other hand, fresh
weight of plants in this treatment was reduced. This

reduction probably accounts for the increase in the concentrations of protein per gram fresh tissue. The reduction in fresh and dry weights may be due to inhibited photosynthesis and speedy depletion of reserve carbohydrates in the intermediary metabolism.

No experimental results are available to elucidate the effects of triazines on the protein metabolism of plants. Several investigators (10, 40, 82, 94, 119) have mentioned that 2,4-D increased the protein content of shoots of different plants. The results of this investigation are contradictory to the results reported for 2,4-D. The mode of action of triazines and 2,4-D herbicides apparently is different. The reason for this difference will be discussed later.

The results of the analysis of particulate proteins show that the chloroplast proteins are affected severely by simazine treatment. Reduction in the concentration of chloroplast proteins began at the initiation of the experiment. On the other hand, the concentrations of mitochondrial, microsomal and cytoplasmic proteins increased in the first two samples. In the third and fourth samples, these proteins also were decreased. The maximum decrease was 61 per cent in the chloroplast proteins in the sample analyzed 12 days after treatment. Thus, this experiment demonstrated that simazine affects chloroplast proteins and subsequently the other proteins.

Ashton et al. (5, 6), in their histological studies of bean shoots, observed alteration of chloroplast

structure by atrazine treatment. In the late stages of phytotoxicity, they found disintegration of chloroplast structure. The major components of chloroplasts are protein and lipids. The finding in the present investigation that simazine primarily affects chloroplast protein may be one reason for the destruction and disintegration of chloroplasts.

The results of the C^{14}-leucine incorporation experiments provide further evidence for the influence of simazine on protein metabolism. The incorporation rate of C^{14}-leucine was slightly enhanced in the total protein content of tissue from plants six days old from 0 and 2.0 lbs. treatment. In the same experiment, great inhibition in the incorporation rate was found in the chloroplast proteins. The incorporation rate of radioactive leucine in total protein was decreased in tissues from plants 10 days old from the 2.0 lbs. treatment. In this sample, the incorporation of C^{14}-leucine in the chloroplast proteins decreased to a greater extent than in the first sample. These results further support the previous findings that simazine inhibits protein synthesis and this inhibition is more pronounced in the chloroplast proteins.

Instead of adding simazine pre-emergence, it was added in the incubation mixture for the oat shoots 10 days old and the C^{14}-leucine incorporation in total and chloroplast proteins was determined. The results

of this experiment were similar to the previous radio-
isotope experiment. However, the differences in the
incorporation rates of plants from 0 and 2.0 lbs.
treatment were not distinct. The low solubility of
simazine may be the reason for this discrepancy in
results. Davis et al. (28) and Schneider (93) reported
that simazine and other triazines are readily absorbed
by the roots. Several workers (28, 38, 39) demonstrated
negligible foliar absorption of simazine.

The primary mode of action of triazines has
been reported by various investigators as the inhibition
of photosynthesis (42, 71, 91 130). However, Ashton
and Uribe (7) concluded from their experiments that
atrazine not only blocks photosynthesis but also inter-
feres with other metabolic processes independent of
photosynthesis. Based upon the results of these several
workers, two experiments were planned to determine if
inhibition in protein synthesis is affected by inhibition
in photosynthesis, or if it is independent of the photo-
synthetic block.

Glucose was supplied in the incubation mixture
for tissues from 0 and 2.0 lbs. simazine rates and the
total and chloroplast proteins were analyzed for C^{14}-
leucine incorporation. The incorporation rate of C^{14}-
leucine in the total and chloroplast proteins of simazine
treated tissue was improved by the addition of glucose in
incubation mixture, but the incorporation rate was not
normal as in 0 rate.

These results suggest that photosynthetic inhibition affects protein synthesis. Since the incorporation rate was not normal in the treatments including glucose, simazine probably may also affect protein synthesis directly.

In another experiment, photosynthesis was blocked by simazine and dark treatments and the incorporation of C^{14}-leucine in total protein of these tissues was determined. The inhibition in leucine incorporation was slightly greater due to dark than simazine treatment. These results further support the findings that the inhibition of protein synthesis is caused by abnormalities in photosynthesis in plant response to simazine.

The free amino acid content decreased simultaneously with an increase in the concentration of protein in plants from the 2.0 lbs. simazine treatment six and eight days after treatment. In the third and fourth samples analyzed 10 and 12 days after treatment, the concentration of free amino acids increased and the protein content decreased.

These findings indicate that simazine does not inhibit the synthesis of free amino acids. The decrease and subsequent increase in amino acids concentration probably were due to the utilization of free amino acids in protein synthesis. Zweig and Ashton (130) concluded from their experiments that aspartic and glutamic acids

are not affected by atrazine phytotoxicity. Other amino acids may be synthesized from these amino acids by transamination mechanism.

The results of this investigation indicate that RNA synthesis is affected by simazine treatment in the same way as protein synthesis. The RNA per gram fresh and dry tissue in plants from 0.5 lb. treatment decreased in all four samples. The RNA content per gram of fresh tissue in plants from 2.0 lbs. simazine treatment increased in the first two samples and decreased in later samples. In the first and second samples, the differences between the RNA content per gram dry tissue in plants from 0 and 2.0 lbs. simazine treatment were not distinct. In the later samples, the RNA content per gram dry tissue decreased in plants from 2.0 lbs. simazine treatment. No noticeable differences were observed in per plant RNA content in different treatments in the first sample, but it decreased in the other three samples.

The increase in RNA content in the tissues from 2.0 lbs. simazine treatment collected six and eight days after treatment may be due to the fact that there was no change in the net concentration of RNA in this treatment, whereas the fresh weight was decreased. The pattern of changes in RNA concentration was similar to changes in protein contents. This relationship indicates that changes in protein contents are due to the changes in RNA concentrations.

Several investigators (10, 62, 86, 119) reported that 2,4-D increased the concentrations of RNA of plant shoots. The results of the reported investigation are not in the line of the results reported for 2,4-D.

The results of the study of ribosomal RNA indicate that simazine influenced RNA contents of all sizes of ribosomes. The reduction in the RNA content of ribosomes was in the order of polysome, $(70S)_2$ and $(70S)_3$. There were slight differences in the ratios of different sizes of ribosomes with monosomes. The ratio was more reduced in polysomes followed by $(70S)_3$ and $(70S)_2$. The rate of incorporation of P^{32} in the ribosomal RNA was almost completely stopped in tissues from 2.0 lbs. simazine treatment. The results of P^{32} incorporation study suggest that the synthesis of ribosomal RNA is completely inhibited by simazine treatment.

The differences in the influence of 2,4-D and simazine herbicides on protein and RNA metabolism indicate that the mode of action of these herbicides are different. van Overbeek (81) commented,"2,4-D causes abnormal growth whereas simazine upsets photosynthesis". He further added, "this extra growth induced by 2,4-D herbicide is caused by a production of extra RNA". The production of extra RNA synthesizes extra protein.

SUMMARY AND CONCLUSIONS

Seminole variety of oats were grown under
controlled temperature, photoperiod and light intensity
in sand with the use of Hoagland-Arnon nutrient solution.
Simazine was added to sand as pre-emergence treatment.
Samples of oat shoots were collected at two-day inter-
vals beginning six days and terminating 12 days after
treatment. Growth studies included the observation of
phytotoxicity symptoms and the determination of fresh
and dry weights. Determinations were made of total
protein, particulate proteins, total RNA, ribosomal RNA,
chlorophylls and free amino acids. The influence of
simazine on protein synthesis was studied by the use of
C^{14}-leucine incorporation and on ribosomal RNA synthesis
by the use of P^{32}.

The phytotoxic symptoms of simazine appeared
in the form of yellowing of leaves by the sixth day
after treatment. Necrosis later began in the leaf tips
and rapidly spread throughout. Plants died in most
cases 14 days after the application of simazine.

Slight increases occurred in the fresh weight
of plants from 0.5 lb. simazine treatment in the first
sample, but fresh and dry weights of plants from 0.5

80

and 2.0 lbs. simazine treatment were decreased in the samples collected 8, 10 and 12 days after treatment.

Simazine reduced the concentrations of chlorophyll a and chlorophyll b in all four samples. In the earlier two samples, chlorophyll a content was reduced more than chlorophyll b, but this finding was reversed 12 days after treatment.

Total protein content was reduced in oat plants from the 0.5 lb. simazine treatment in all four samples. The 2.0 lbs. rate of simazine caused an increase in total protein content per gram fresh tissue in plants analyzed six and eight days after treatment. In later samples, the protein content in this treatment decreased.

The influence of simazine on total RNA was similar to that on protein. RNA content of plants from 0.5 lb. simazine treatment was decreased in all samples. The concentration of RNA per gram fresh tissue in plants from the 2.0 lbs. treatment increased in the first and second samples and decreased in others. However, RNA per plant in this treatment slightly increased in the first sample and decreased in other three samples. Dry weight determinations of RNA showed no differences in the first and second samples of plants from the 0 and 2.0 lbs. treatment, but a reduction occurred in the third and fourth samples of plants from the 2.0 lbs. treatment.

Simazine caused a decrease in the concentrations of free amino acids in the first two samples and an

increase in the later samples.

Chloroplast proteins were reduced in plants
from the 2.0 lbs. simazine treatment in all samples.
However, the concentrations of mitochondrial, micro-
somal and cytoplasmic proteins in this treatment
increased in the first two samples but decreased in
samples collected 10 and 12 days after treatment.

Simazine caused a slight increase in the rate
of incorporation of leucine in total protein of tissues
collected six days after treatment. The rate of
C^{14}-leucine incorporation in total protein decreased
in tissues collected 10 days after the application of
simazine. Whereas the rate of C^{14}-leucine incorporation
in chloroplast proteins of plants from the 2.0 lbs.
treatment decreased in both samples.

Addition of 6 per cent glucose to C^{14}-leucine
improved the incorporation rate of radioactive leucine
in total and chloroplast proteins of plants from 2.0 lbs.
simazine treatment. The incorporation rate of C^{14}-
leucine in total protein of plants from 2.0 lbs. simazine
treatment was slightly higher in comparison to the incor-
poration rate of etiolated tissues from 0 lb.

The analyses of RNA of different sizes of ribo-
somes indicated that simazine affected all sizes of
ribosomes. The incorporation of P^{32} in ribosomal RNA
was almost completely blocked in the plants from 2.0 lbs.
simazine treatment.

Based on results of this investigation, the following conclusions appear justified concerning the influence of simazine applied pre-emergence to oats:

1. The phytotoxic symptoms of simazine appear six days after treatment in the form of yellowing of leaves followed by necrosis of leaf tips. The plants die 14 days after treatment.

2. Simazine causes decrease in fresh and dry weights of shoots.

3. The concentrations of chlorophyll a and chlorophyll b are decreased by simazine treatment.

4. Simazine decreases concentrations of free amino acids in plants six and eight days after treatment, but these concentrations are increased by the tenth day.

5. Simazine causes a decrease in total protein per plant eight days after the application of simazine.

6. Reduction in the concentration of chloroplast proteins begins as early as six days after simazine treatment. The concentrations of mitochondrial, microsomal and cytoplasmic proteins increase in plants through the eighth day after treatment and then decreases.

7. Simazine inhibits incorporation of C^{14}-leucine in chloroplast proteins. The incorporation rate of C^{14}-leucine in total protein increases slightly six days after treatment but decreases after another four days.

8. Glucose improves the rate of incorporation of radioactive leucine in total and chloroplast proteins of plants from simazine treatment.

9. Simazine causes a decrease in total RNA per plant eight days after simazine treatment.

10. Simazine causes reduction in RNA contents in all sizes of ribosomes.

11. The rate of incorporation of P^{32} in ribosomal RNA is almost completely blocked due to simazine treatment.

BIBLIOGRAPHY

1. Akers, T. J. and S. C. Fang. 1956. Studies on plant metabolism. VI. Effect of 2,4-D on the metabolism of aspartic acid and glutamic acid in the bean plant. Plant Physiol. 31:34-37.

2. Allen, W. S. and R. D. Palmer. 1963. The mode of action of simazine in barley. Weeds 11:27-30.

3. Ashton, F. M. 1962. Action spectra of atrazine injury. Plant Physiol (suppl.) 35:XXV.

4. _____ and T. Bisalputra. 1964. Effect of atrazine on Chlorella. Plant Physiol. (suppl.) 39:XXXII.

5. _____, E. M. Gifford, Jr. and T. Bisalputra. 1963. Structural changes in Phaseolus vulgaris induced by atrazine. I. Histological changes. Bot. Gaz. 124:329-335.

6. _____, _____ and _____. 1963. Structural changes in Phaseolus vulgaris induced by atrazine. II. Effects on fine structure of chloroplasts. Bot. Gaz. 125:336-343.

7. _____ and E. G. Uribe. 1962. Effect of atrazine on sucrose-C^{14} and serine-C^{14} metabolism. Weeds 10:295-297.

8. _____, G. Zweig and G. W. Mason. 1960. The effect of certain triazines on $C^{14}O_2$ fixation in red kidney beans. Weeds 8:448-451.

9. Association of Official Agricultural Chemists. 1955. Washington, D.C. 123 p.

10. Basler, E. and K. Nakazawa. 1960. Some effects of 2,4-dichlorophenoxyacetic acid on nucleic acids of cotton cotyledon tissue. Plant Physiol. (suppl.) 35:XXVIII.

11. Benson, A. F., J. F. G. M. Wintermans and R. Wiser. 1959. Chloroplast lipids as carbohydrate reservoirs. Plant Physiol. 34:315-317.

12. Bernlohr, R. W. and G. C. Webster. 1958. Transfer of oxygen-18 during amino acid activation. Arch. Biochem. Biophys. 73:276-278.

13. Bishop, J., E. Allen, J. Leahy, A. Morris and R. Schweet. 1960. Stages in hemoglobin synthesis in a cell-free system. Federation Proc. 19:346.

14. Biswas, P. K. 1964. Absorption, diffusion and translocation of C^{14}-labeled herbicides by peanut leaves. Weeds 12:31-32.

15. Brawermann, G. and E. Charagaff. 1959. Changes in protein and ribonucleic acid during the formation of chloroplast in Euglena gracillis. Biochim. et Biophys. Acta 31:164-171.

16. British Weed Control Council. 1960. Weed Control Handbook, (Ed. E. K. Woodford). Blackwell Scientific Publications, Oxford (England). 264 p.

17. Brown, G. L. and G. Zubay. 1960. Physical properties of soluble RNA of Escherichia coli. J. Mol. Biol. 2:287-296.

18. Buchholtz, F. P. 1963. Use of atrazine and other triazine herbicides in control of quackgrass in corn fields. Weeds 11:202-207.

19. Burnside, O. C. and R. Behrens. 1961. Phytotoxicity of simazine. Weeds 9:154-157.

20. Butler, J. A. V., P. Cohn and P. Simson. 1960. The presence of basic proteins in microsomes. Biochim. et Biophys. Acta 38:386-388.

21. Castelfranco, P. and M. Brown. 1962. Purification and properties of the simazine-resistant factor of Zea mays. Weeds 10:131-135.

22. _____, C. L. Foy and D. B. Deutsch. 1961. Non-enzymatic detoxification of 2-chloro-4,6-bis (ethylamino)-s-triazine (simazine) by extracts of Zea mays. Weeds 9:580-591.

23. Chrispeels, M. J. and J. B. Hanson. 1962. The increase in ribonucleic acid content of cytoplasmic particulates of soybean hypocotyl induced by 2,4-dichlorophenoxyacetic acid. Weeds 10:123-125.

24. Cole, R. D., J. Coote and T. S. Work. 1957. Activation of amino acids by soluble enzymes from pancreas and other tissues. Nature 179:199.

25. Crafts, A. S. 1960. The Chemistry and Mode of
    Action of Herbicides. Interscience Publishers,
    Inc., New York. 104-125 p.

26. Crick, F. H. C. 1958. On protein synthesis.
    Symp. Soc. Exptl. Biol. XII:138-163.

27. Crombie, W. M. 1958. Fatty acids in chloroplasts
    and leaves. J. Exptl. Bot. 9:254-261.

28. Davis, D. E., H. H. Funderburk, Jr. and N. G. Sansing.
    1959. The absorption and translocation of C^{14}-labeled
    simazine by corn, cotton and cucumber. Weeds 7:300-309.

29. Davis, J. W. and G. D. Novelli. 1958. The activation
    of amino acids in extracts of pea seedlings. Arch.
    Biochem. Biophys. 75:299-308.

30. DeMoss, J. A. and G. D. Novelli. 1956. An amino acid
    dependent exchange between P^{32} labeled inorganic
    pyrophosphate and ATP in microbial extracts. Biochim.
    et Biophys. Acta 22:49-61.

31. _____, S. M. Genuth and G. D. Novelli. 1956.
    The enzymatic activation of amino acids via their
    acyladenylate derivatives. Proc. Natl. Acad. Sci.
    U. S. 42:325-332.

32. Dunham, R. S. 1951. General responses in crop plants.
    In Plant Growth Substances, (Ed. F. Skoog). 195-206 p.

33. Eastin, E. F., R. D. Palmer and C. O. Grogan. 1964.
    Mode of action of atrazine and simazine in susceptible
    and resistant lines of corn. Weeds 12:49-52.

34. Exer, B. 1958. Über Pflanzenwachstumsregulatoren.
    Der Einfluss von simazin auf den Pflanzenstoffwechsel.
    Experientia 14:135.

35. _____. 1961. Hemung der hillrektion durch
    herbizide. Weed Res. 1:233-244.

36. Fink, R. J. and O. H. Fletchall. 1963. Forage estab-
    lishment in soil containing atrazine or simazine residues.
    Weeds 11:81-83.

37. Foy, C. L. 1962. Accumulation of s-triazine derivatives
    in the lysigenous glands of Gossypium hirsutum L. Plant
    Physiol. (suppl.) 37:XXV.

38. _____. 1964. Volatility and tracer studies with
    alkylamino-s-triazines. Weeds 12:103-108.

39. _____ and P. Castelfranco. 1961. Distribution
    and metabolic fate of C^{14}-labeled 2-chloro-4,6-bis
    (ethylamino)-s-triazines in relation to phytotoxicity.
    Plant Physiol. (suppl.) 35:XXVIII.

40. Freiberg, S. R. and H. E. Clark. 1955. Changes in
    nitrogen fractions and proteolytic enzymes of soybean
    plant treated with 2,4-dichlorophenoxy-acetic acid.
    Plant Physiol. 30:39-46.

41. Frey-Wyssling, A. 1953. The submicroscopic Morphology
    of Protoplasm and its Derivatives. Elsevier Publishing
    Co., Amsterdam (Holland). 243-262 p.

42. Funderburk, H. H. Jr. and D. E. Davis. 1963. Metabolism
    of C14-chain and ring labeled simazine by several other
    plants. Proc. SWC. 16:387.

43. _____ and _____. 1963. The metabolism of
    C14-chain and ring labeled simazine by corn and the
    effect of atrazine on plant respiratory enzymes.
    Weeds 11:101-104.

44. Fults, J. L. and M. G. Payne. 1956. Effect of
    2,4-dichlorophenoxyacetic acid and maleic hydrazide
    on free amino acids and proteins in potato, sugar beet
    and bean tops. Bot. Gaz. 118:130-133.

45. Gast, A. 1958. Über Pflanzenwachstumsregulatoren.
    Beitrage zur Kenntris der Phytotosischen Wirkung von
    Triazinen. Experientia 14:134.

46. Gierer, A. 1963. Function of aggregated reticulocyte
    ribosomes in protein synthesis. J. Mol. Biol. 6:148-157.

47. Gigger, R. P. and J. H. Flangan. 1960. A report on
    atrazine post-emergence in corn, weed control and corn
    plant residue analysis. Proc. NEWCC. 14:231-236.

48. Good, N. E. 1961. Inhibitors of Hill reaction.
    Plant Physiol. 36:788-803.

49. Grossi, L. G. and K. Maldave. 1959. Transfer of
    RNA-bound amino acids to microsomal proteins. Biochim.
    et Biophys. acta 35:275-277.

50. Grover, R. 1962. Uptake and distribution of simazine-C14
    and propazine-C14 in some tree seedlings. Plant Physiol.
    (suppl.) 37:XII.

51. Gysin, H. and E. Knusli. 1960. Chemistry and herbicidal
    properties of triazine derivatives. Advances in Pest
    Control Res. 3:289-358.

52. Hamilton, R. H. 1964. A corn mutant deficient in
    2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one with an
    altered tolerance of atrazine. Weeds 12:27-30.

53. _____, R. S. Bandurski and W. H. Reisch. 1962. Isolation and characterization of a cyclic hydroxamate from Zea mays. Cereal Chem. 39:107-113.

54. _____, and D. E. Moreland. 1962. Simazine: Degradation by corn seedlings. Science 135:373-374.

55. Hill, R. and C. P. Whittingham. 1955. Photosynthesis. Methren and Co., Ltd., London (England). 164 p.

56. Hoagland, D. R. and D. I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Expt. Sta. Cir. 347.

57. Hogland, M. B. 1955. An enzymatic mechanism for amino acid activation in animal tissues. Biochim. et Biophys. Acta 16:288-289.

58. _____, M. L. Stephenson, J. F. Scott, L. I. Hencht and P. C. Zamecnik. 1958. A soluble ribonucleic acid intermediate in protein synthesis. J. Biol. Chem. 231:241-257.

59. _____, P. C. Zamecnik, N. Sharon, F. Lipman, M. P. Stulberg and P. D. Boyer. 1957. Oxygen transfer to AMP in the enzymatic synthesis of the hydroxamate of tryptophan. Biochim. et Biophys. Acta 26:215-217.

60. Jacob, F. and J. Monod. 1961. Genetic regulatory mechanisms in the synthesis of protein. J. Mol. Biol. 3:318-356.

61. Key, J. L. 1963. Studies on 2,4-D induced changes in ribonucleic acid metabolism in excised corn mesocotyl tissue. Weeds 11:177-180.

62. _____ and J. B. Hanson. 1961. Some effects of 2,4-dichlorophenoxyacetic acid on soluble nucleotides and nucleic acid of soybean seedlings. Plant Physiol. 36:145-152.

63. Krinsky, N. I. and T. H. Goldsmith. 1960. The carotenoids of the flagellated alga, Euglena gracilis. Arch. Biochem. Biophys. 91:271-279.

64. Krishnaswamy, P. R. and A. Meister. 1960. Enzymatic synthesis and reaction of amino acyl adenylates. J. Biol. Chem. 235:408-415.

65. Lipman, F. 1958. Some facts and problems. Proc. Natl. Acad. Sci. U.S. 44: 67-72.

66. Lowry, O. H., N. J. Rosenbrough, A. L. Farr and
    R. J. Randall. 1951. Protein measurement with the
    Folin-Phenol reagent. J. Biol. Chem. 193:265-275.

67. Menke, W. 1963. Experiments made to elucidate
    the molecular structure of chloroplast. In
    Photosynthetic Mechanism of Green Plants. Symp.
    Natl. Acad. Sci. U.S. 537-544 p.

68. McQuillen, K., R. B. Roberts and R. J. Britten.
    1959. Synthesis of nascent protein by ribosomes
    in Escherichia coli. Proc. Natl. Acad. Sci. U.S.
    45:1437-1449.

69. Moldave, K. and C. G. Grossi. 1960. Studies on
    the nucleotide requirement in the transfer of
    amino acids from soluble RNA to microsomal proteins.
    Federation Proc. 19:351.

70. Montgomery, M. and V. H. Freed. 1961. The uptake,
    translocation and metabolism of simazine and atrazine
    by corn plants. Weeds 9:231-237.

71. Moreland, D. E. 1959. Studies on the mechanism of
    herbicidal action of 2-chloro-4,6-bis(ethylamino)-
    s-triazine. Plant Physiol. 34:432-435.

72. _____ and K. L. Hill. 1962. Interference of
    herbicide with the Hill reaction of isolated chloro-
    plast. Weeds 10:229-235.

73. Nathans, D. and W. C. Hilsman. 1960. Purification
    of a supernatant factor required for transfer of sRNA-
    amino acid to liver microsomes. Federation Proc.
    19:347.

74. Niremberg, M. W. and J. H. Mathaei. 1961. Character-
    istics and stabilization of DNAase-sensitive protein
    synthesis in E.coli extracts. Proc. Natl. Acad. Sci.
    U.S. 47:1580-1587.

75. _____ and _____. 1961. The dependence of
    cell free protein synthesis in E.coli upon naturally
    occurring synthetic polyribonucleotides. Proc. Natl.
    Acad. Sci. U.S. 47:1588-1601.

76. Nishman, B. 1959. Incorporation and activation of
    amino acids by disrupted protoplasts of Escherichia
    coli. Biochim. et Biophys. Acta 32:18-31.

77. _____, F. Bergmann and P. Berg. 1957. Obser-
    vations on amino acid-dependent exchanges of inorganic
    pyrophosphate and ATP. Biochim. et Biophys. Acta
    26:639-640.

78. Novelli, G. D. 1958. Some problems concerning the
    activation of amino acids. Proc. Natl. Acad. Sci.
    U.S. 44:816-892.

79. Ogur, M. and G. Rosen. 1950. The nucleic acids of
    plant tissue. I. The extraction and estimation of
    deoxypentose nucleic and pentose nucleic acid. Arch.
    Biochem. Biophys. 25:262-276.

80. Okamoto, T. and M. Takanami. 1963. Interactions of
    ribosomal natural polynucleotides. Biochim. et
    Biophys. Acta 76:266-274.

81. Overbeek, van T. 1964. Survey of mechanism of
    herbicide action. In The Physiology and Biochemistry
    of Herbicides, (Ed. L. J. Audus). Academic Press,
    New York. 387-399 p.

82. Payne, M. G., J. L. Fults and R. J. Hay. 1952. The
    effect of 2,4-D treatment on free amino acids in
    potato tubers. Amer. Potato J. 29:142-150.

83. Peters, R. A. 1957. Notes on simazine as an herbi-
    cide on corn compared with several other materials.
    Proc. NEWCC. 11:283-285.

84. Plaisted, P. H. and D. P. Ryskiewich. 1962. The
    uptake and metabolism of C^{14}-labeled simazine by
    young corn plants. Plant Physiol. (suppl.) 37:XXV.

85. Ragab, M. T. H. and J. P. McCollum. 1961. Degra-
    dation of C^{14}-labeled simazine by plants and soil
    microorganism. Weeds 9:72-84.

86. Rebstock, T. L., C. L. Hamner and H. M. Sell. 1954.
    The influence of 2,4-dichlorophenoxyacetic acid on
    the phosphorus metabolism of cranberry bean plants
    (Phaseolus vulgaris). Plant Physiol. 29:490-491.

87. Risebrough, R. W., A. Tissieres and J. D. Watson.
    1962. Messenger-RNA attachment to active ribosomes.
    Proc. Natl. Acad. Sci. U.S. 48:430-436.

88. Roberts, R. B., R. J. Britten and E. T. Bolton.
    1958. Fractionation of Escherichia coli for kinetic
    studies. In Microsomal Particles and Protein
    Synthesis, (Ed. R. B. Roberts). Pergamon Press,
    New York. 84-94 p.

89. Rodgers, E. G. and M. Wilcox. 1963. Leaching
    characteristics of certain herbicides in selected
    soils. Fla. Agrl. Expt. Sta. Annu. Rept. 60 p.

90. Roth, M. W. 1957. Etude comparee de la reaction du Mais et du Ble a la simazin, substance herbicide. Compt. Rend. Acad. Sci. 10:942-944.

91. _____. 1958. Plant growth regulators. The effect of simazine on the physiology of Elodea. Experientia 14:137-138.

92. Schirman, R. and K. P. Buchholtz. 1960. The effect of atrazine on the carbohydrate content levels in rhizomes of quackgrass. Proc. NCWCC. 17:19.

93. Schneider, E. O. 1959. A discussion of the mode of action, tolerance and soil type effects of the triazines. Proc. NEWCC. 13:416-420.

94. Sell, H. M., R. W. Luecke, B. M. Tayler and C. L. Hamner. 1949. Changes in chemical composition of the stems of red kidney bean plants treated with 2,4-dichlorophenoxyacetic acid. Plant Physiol. 24:295-299.

95. Sheets, T. J. 1961. Uptake and distribution of simazine by oat and cotton seedlings. Weeds 9:1-13.

96. _____. 1961. Toxicity of simazine to seedling oat plants. Weeds 9:331-333.

97. Shigeura, H. T. and E. Chargaff. 1960. Action of ribonuclease on a microsomal ribonucleoprotein. Biochim. et Biophys. Acta 37:347-349.

98. Sissakin, N. M. 1958. Enzymology of the plastids. Adv. in Enzymol. 20:201-236.

99. Spencer, M., W. Fuller, M. H. F. Wilkins and G. L. Brown. 1962. Determination of helical configuration study of crystalline amino acid transfer ribonucleic acid. Nature 194:1014-1020.

100. Springer, F. B., Jr. 1960. The effectiveness of simazine in 1959. Proc. NEWCC. 14:237-240.

101. Smith, D. and K. P. Buchholtz. 1962. Transpiration rate reduced in plants with atrazine. Science 136:263-264.

102. Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York. 194-229 p.

103. Thomas, J. B., L. C. Post and N. Vetregt. 1954. Localization of chlorophyll within the chloroplast. Biochim. et Biophys. Acta 13:20-30.

104. Tissieres, A. 1960. Some properties of soluble ribonucleic acid from Escherichia coli. J. Mol. Biol. 1:365-374.

105. _____, D. Schlessinger and F. Gros. 1960. Amino acid incorporation into proteins by Escherichia coli ribosomes. Proc. Natl. Acad. Sci. U.S. 46:1450-1462.

106. _____, J. D. Watson and D. Schlessinger. 1959. Ribonucleoprotein particles from Escherichia coli. J. Mol. Biol. 1:221-233.

107. Trurnit, H. H. 1958. The architecture of the chloroplast in relation to its photosynthetic activities (Discussion). Brookhaven Symposia in Biol. 11:101-116.

108. T'SO, P., J. Bonner and H. Dintzis. 1958. On the similarity of amino acid composition of microsomal nucleoprotein particles. Arch. Biochem. Biophys. 76:225-227.

109. van Oorschot, J. L. P. and M. Belksma. 1961. A assembly for the continuous recording of CO_2 exchange and transpiration of whole plants. Weed Res. 1:245-257.

110. Wahba, A. J., C. Basilo, J. F. Speyer, P. L. Lengyrel, R. S. Miller and S. Ochoa. 1962. Synthetic poly-nucleotides and the amino acid code. IV. Proc. Natl. Acad. Sci. U.S. 48:1683-1686.

111. Wakonig, R. and T. J. Arnason. 1959. Chromosome breakage induced in Vicia faba root tips by 2,4,6-tri (ethylimino)-1,3,5-triazine. Can. J. Bot. 37:403.

112. Warner, J. R., A. Rich and C. E. Hall. 1962. Electron microscope studies of ribosomal clusters synthesizing hemoglobin. Science 138:1399-1403.

113. _____, P. M. Knopf and A. Rich. 1963. A multiple ribosomal structure in protein synthesis. Proc. Natl. Acad. Sci. U.S. 49:122-129.

114. Webster, G. C. 1958. Activation of amino acids and amides by cell-free preparations. Arch. Biochem. Biophys. 82:125-134.

115. _____. 1959. Protein synthesis by isolated nucleoprotein particles. Arch. Biochem. Biophys. 85:159-170.

116. _____. 1960. Protein synthesis from amino acids associated with ribonucleic acid. Arch. Biochem. Biophys. 89:53-58.

117. _____ .and J. B. Lingrel. 1961. Protein
synthesis by isolated ribosomes. In The Bio-
synthesis of Protein, (Ed. R. J. C. Harris).
Academic Press, New York. 301-318 p.

118. Weller, L. E., C. D. Ball and H. M. Sell. 1957.
Studies of maleic hydrazide interactions with
thiol compounds. Plant Physiol. 32:140-147.

119. West, S. H., J. B. Hanson and J. L. Key. 1960.
Effect of 2,4-dichloro-phenoxyacetic acid and
protein content of seedling tissue. Weeds 8:333-340.

120. _____ and J. B. Hanson. 1960. The degradation
of isolated microsomes from cucumber and corn
seedlings. Weeds 8:341-348.

121. Wills, G. D., D. E. Davis and H. H. Funderburk, Jr.
1963. The effect of atrazine on transpiration in
corn, cotton and soybean. Weeds 11:253-255.

122. Wolf, F. T., J. G. Coniglio and J. T. Davis. 1962.
Fatty acids of spinach chloroplasts. Plant Physiol.
37:83-85.

123. Wolken, J. J. 1956. A molecular morphology of
Euglena gracilis var. bacillaris. J. Protozool.
3:211-221.

124. _____ . 1959. The structure of the chloroplast.
Ann. Rev. Plant Physiol. 10-71-86.

125. _____ . 1961. Euglena: An Experimental Organism
for Biochemical Studies. Rutgers Uni. Press, New
Brunswick, New Jersey. 111-125 p.

126. _____ . 1963. The chloroplast structure in
photosynthesis. In Photosynthetic Mechanism of
Green Plants. Symp. Natl. Acad. Sci. U.S. 575-587 p.

127. _____ and F. A. Schwertz. 1953. Chlorophyll
monolayers in chloroplasts. J. Gen. Physiol. 37:111-119.

128. Yoshida, A. and T. Tobita. 1960. Studies on the
mechanism of protein synthesis. Non uniform incor-
poration of C^{14}-leucine into α-amylase and the
presence of α-amylase precursor. Biochim. et
Biophys. Acta 37:513-520.

129. Zamecnik, P. C. 1962. Unsettled question in the
field of protein synthesis. Biochem. J. 85:257-264.

130. Zweig, G. and F. M. Ashton. 1962. The effect of
2-chloro-4-ethylamino-6-isopropylamino-s-triazine
(atrazine) on distribution of C^{14}-compounds following
$C^{14}O_2$ fixation in excised kidney bean leaves. J.
Exptl. Bot. 13:5-11.

131. Zubay, G. 1963. Molecular model for protein synthesis. Science 140:1092–1094.

BIOGRAPHICAL SKETCH

The author, Raghvendra Pal Singh, was born on January 10, 1939, in the district of Basti, U.P., India. He passed his high school and intermediate examinations of Uttar Pradesh Board of High School in June 1953 and 1955, respectively. In 1955, he was transferred to Government Agriculture College, Kanpur (Agra University), from which he received the degrees of Bachelor of Science in Agriculture, in 1957, and Master of Science in Agriculture, with Agronomy as a major subject, in 1959.

He entered the University of Florida, Gainesville, in February 1962. He completed his work toward the degree of Doctor of Philosophy in the Department of Agronomy in December 1964.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1964

M. A. Brooker
Dean, College of Agriculture

Supervisory Committee:

E. B. Rodgers
Chairman

Shirlie H. West
Co-chairman

J. E. Humphreys

M. Wilson

V. F. Nettles

_____
Dean, Graduate School